Antithrombotic Therapy

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Antithrombotic Therapy

By PAUL W BOYLES, M D

Instructor in Medicine University of Miami School of Medicine Jackson Memorial Hospital Miami Florida and Director Coagulation Research Laboratory Miami Heart Institute Miami Beach Florida



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Contents

PREFACE vii

1 THE MECHANISM OF BLOOD COAGULATION 1

2 CLOTTING TESTS 8

3 ANTITHROMBOTIC REACTIONS 15

4 CLINICAL USE OF ANTITHROMBOTIC AGENTS 21

5 PRESENT STATUS OF ANTICOAGULANT THERAPY 40

6 LONG TERM ANTICOAGULANT THERAPY 50

7 THROMBOLYTIC THERAPY 55

BIBLIOGRAPHY 80

ADDENDUM 95

SYNONYMS FOR BLOOD COAGULATION FACTORS 95

TECHNIC OF BLOOD COAGULATION TESTS 98

SOLUTIONS 122

INDEX 127

Preface

To invade two such controversial fields of medicine as the mechanism of blood clotting and the treatment of thromboembolic diseases would seem presumptuous However rapid progress has been made in both these fields in the last 10 years Increased understanding of the complex mechanisms involved in blood coagulation has been made possible through the study of purified coagulation proteins With this technical advance in the purification of these various factors a new era in the direct treatment of thromboembolic diseases is now possible through the clinical utilization of streptokinase and fibrinolysin These agents combined with anticoagulants offer a rational approach to the treatment of intravascular clotting This monograph has been prepared to provide the clinician with a practical understanding of the normal mechanisms of blood coagulation and coagulation defects produced by antithrombotic therapy Perhaps in no other field of medicine is the practical application of basic research and laboratory studies so vital to the patient as in the present use of antithrombotic agents in thromboembolic diseases

The encouragement and suggestions of my colleagues and friends I gratefully acknowledge Doctors E S Nichol Ralph Jones Jr Franz H Stewart and John H Ferguson Also I gratefully acknowledge the assistance of Mrs Catherine Ashley Florence Wey Tracy Channing Fox and Lillian Gong

Paul W Boyles M D

To

Chris, Pat and Marc

To

Chris, Pat and Marc

1 *The Mechanism of Blood Coagulation*

THE LITERATURE on blood coagulation contributes little because of conflicting claims superfluous hypotheses and varied terminology The basic facts however are relatively simple Blood coagulation is essentially the conversion of a soluble plasma protein (fibrinogen) into an insoluble colloidal gel (fibrin) This is specifically influenced by an enzyme (thrombin) [1] which is formed from an inactive plasma protein (prothrombin) The activation of prothrombin is catalyzed by thromboplastin an agent whose chemical nature is still unknown Substances having thromboplastic activity are present in various tissues particularly brain lung uterus and endothelium Plasma or intrinsic thromboplastin is produced by an interaction of coagulation proteins with phospholipids derived from platelets or from crude extracts of animal or vegetable origin [38]

Initiating the coagulation process is injury to the endothelium which then forms a foreign surface that activates intrinsic thromboplastin Also if rupture of vessels occurs tissue thromboplastin enters the blood to initiate the process further Local factors such as stasis [76] narrowing of vessels from atherosclerosis or tissue damage determine the progression of clotting since the coagulation factors which are locally activated are also rapidly diluted in the circulating blood and quickly inactivated Blood does not clot in normal vessels therefore intravascular clotting usually indicates some local pathologic condition After its initiation the coagulation process can be divided into three major reactions namely the generation of thromboplastin thrombin and fibrin respectively

The overall time required for these reactions is called the clotting time A clinical test commonly used for the velocity

of the last two reactions thrombin and fibrin is called the prothrombin time Naturally occurring inhibitors and the dilutory effects of circulating blood tend to limit the clot locally This may then be dissolved by an enzyme (fibrinolysin) [1] which is present in the plasma in an inactive state The three coagulation reactions the various deficiencies and laboratory tests are discussed individually

STAGE I THROMBOPLASTIN GENERATION

Since calcium ions are necessary for coagulation blood can be rendered incoagulable by the addition of sodium citrate or potassium oxalate which bind the calcium When excess calcium is then added to either citrated or oxalated blood the clotting mechanism is again restored This process is known as recalcification However platelet free plasma prepared by centrifuging citrated or oxalated blood in order to remove the formed elements (red blood cells white blood cells and platelets) still will not coagulate when recalcified

This demonstrates the importance of an additional factor in the hemostatic mechanism which has been proved to be the platelets In fact diathetic bleeding (thrombocytopenic purpura) is seen clinically in patients with a markedly reduced platelet count (below 50 000 per cu mm) Recent studies however have shown that phospholipids containing phosphatidyl ethanolamines can be substituted for platelets both in vitro and in vivo [160] Nevertheless with increasing purification of the phospholipid fractions there is a decrease in the efficacy of phospholipids to replace platelets indicating the existence of a cofactor or alteration of the phospholipid in the purification process which as yet has not been clearly defined

Furthermore platelet free plasma aged for several days at 37 C (body temperature) will not clot even with the addition of both platelets and calcium ions This can be corrected however by freshly drawn platelet free plasma indicating there is a labile factor present in blood necessary for normal hemostasis Hemo

philia is a bleeding disorder in which there is a congenital absence of this factor called antihemophilic globulin (AGH Factor VIII) [19] Concentrates of either antihemophilic globulin or fresh normal plasma will correct the abnormal clotting of hemophilic plasma in vivo and in vitro Antihemophilic globulin is consumed in the clotting process This loss is substantiated by the failure of fresh normal serum to correct the abnormal clotting of hemophilic blood

Until 1953 hemophilia was generally considered to be a specific disease clinically diagnosed because of a familial tendency in males toward frequent episodes of bleeding notably into the joints following minor trauma and also because of prolonged bleeding after tooth extraction or minor bruises Bleeding ceases following the intravenous administration of fresh plasma In 1953 several patients were described with clinical histories typical of hemophilia but the admixture of their blood with other hemophilic blood samples resulted in the mutual correction of abnormal clotting of both samples Since these bloods correct each other it is apparent that the factors deficient in the mixed bloods are not the same Furthermore the serum from a normal person corrects the abnormal clotting in one type of hemophilia but not in the other type Thus it is clear that the clinical syndrome called hemophilia is not a single disease but at least two entities manifested in similar clinical pictures Hemophilia corrected with normal serum is called Christmas disease or plasma thromboplastin component deficiency (PTC Factor IX) [3] AHG and PTC differ in that antihemophilic globulin is labile and consumed in the clotting process whereas PTC is stable and present in the serum

Other diseases have been described in which the clinical picture is basically similar to classic hemophilia and Christmas disease but in which additional different factors are absent Again these factors have been demonstrated by the mutual correction of abnormal clotting by blood from patients known to have either classic hemophilia or Christmas disease with blood from patients with congenital deficiency of these different factors

These findings have given rise to the concept that hemophilia forms a group of conditions with a related clinical picture and specific deficiences of coagulation factors The names applied to these various factors are plasma thromboplastin antecedent (PTA Factor X) [93] Hageman factor and Stuart factor [10] They are similar to the Christmas factor in that they are stable and present in normal serum A characteristic they show with anti hemophilic globulin is that they are all involved in the first stage of coagulation This is demonstrated by the correcting effect of the clotting time with thromboplastin added to any of the deficient bloods

Thromboplastin derived from either tissue extracts or from plasma requires two factors normally present in blood in order to be active [106] One is labile and present in the plasma (Acceler *ator Globulin AcG Factor V Pro accelerin Labile Factor)* the other factor is stable and is principally in the serum (Factor VII Serum Prothrombin Conversion SPCA Convertin Stable Factor) [4] AcG is converted to a more active form during the process of coagulation (Serum Accelerator Globulin Factor VI Accelerin)

Congenital deficiencies of either AcG or SPCA result in hemorrhagic diatheses Thromboplastin extracted from tissues contains varying amounts of the accelerators AcG and SPCA As thrombolplastin ages AcG is destroyed and thromboplastin loses some of its original activity The activity retained how ever is due to the presence of the stable SPCA Anticoagulant therapy with Dicumarol and related compounds causes a depres sion in the level of SPCA and prothrombin which prolongs the one stage prothrombin time [17 1] This occurs regardless of the level of AcG because tissue thromboplastin does not have suffi cient adherent SPCA to activate thromboplastin with enough rapidity to give a normal prothrombin time The amount of contaminating SPCA is variable in different preparations of tissue thromboplastin This variation is commonly overlooked in the determination of the one stage prothrombin time in pa tients given *Dicumarol and may account for some episodes of*

bleeding when the prothrombin time is not exceedingly prolonged This suggests that the thromboplastin used in the determination of the one stage prothrombin time in patients receiving anticoagulants should be free of all contaminating accelerator activity in order to reflect accurately the true level of the coagulation factors as measured by the one stage prothrombin test Accelerator globulin is not affected by Dicumarol and thus is not variable in prothrombin determinations from patients on Dicumarol (See DIAGRAM I for the formation of active thromboplastin)

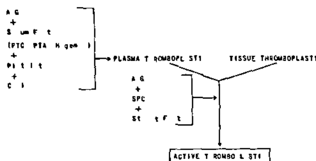


DIAGRAM I—Formation of active thromboplastin

STAGE II THROMBIN FORMATION

Thrombin is formed from prothrombin a protein produced by the liver requiring vitamin K for its synthesis [8 13] Under physiologic conditions both calcium ions and active thromboplastin [94] are required for the conversion which involves the breakdown essentially of *prothrombin into several protein* moieties [119] One of these proteins is thrombin The formation of thrombin is also catalyzed by at least two additional factors accelerator globulin (AcG labile factor Factor V) and serum prothrombin conversion accelerator (SPCA stable factor Factor VII) As stated before AcG and SPCA have similar functions but differ in that AcG is labile and SPCA is stable Both are present in the plasma in relatively inactive forms and in the

serum in active states The active serum form of AcG is called serum AcG Hemorrhagic diathesis occurs when congenital deficiencies exist in prothrombin AcG or SPCA

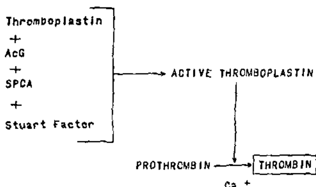


DIAGRAM 2—Stage two reaction in thrombin formation

Indirectly however another factor involved in conversion of prothrombin to thrombin is the Stuart factor which functions with AcG and SPCA in the activation of thromboplastin The Stuart factor is remarkably similar to SPCA but the existence of two separate factors has been proved by diseases in which there is a congenital absence of either factor (See DIAGRAM 2 for the stage two reaction)

STAGE III FIBRIN FORMATION

Purified fibrinogen solutions will not clot on standing or on simple recalcification However trace amounts of thrombin will convert fibrinogen to fibrin The speed of this conversion as in all enzymatically catalyzed reactions is directly related to the thrombin concentration [36, 1] Thrombin alters fibrinogen molecules with subsequent polymerization to fibrin and the



DIAGRAM 3—Stage three reaction in fibrin formation

release of soluble polypeptides Patients congenitally deficient in fibrinogen have been reported and their episodes of bleeding are effectively treated with concentrates of either fibrinogen or normal plasma Dicumarol or heparin does not alter the level of circulating fibrinogen (See DIAGRAM 3 for the stage three reaction)

SUMMARY

Intravascular clot formation represents a disturbance in the normal hemostatic mechanism and is caused by local conditions which initiate coagulation The first phase of clotting results from the interaction of several factors present in the plasma with either platelets or tissue thromboplastin in order to activate thromboplastin Active thromboplastin catalyzes the conversion of prothrombin to thrombin Thrombin then enzymatically acts on the soluble plasma protein fibrinogen to form the insoluble fibrin clot Progressive coagulation of the circulating blood is prevented by naturally occurring inhibitors and by the diluting effects of the blood The final removal of the fibrin clot by the dissolving action of fibrinolysin is fully discussed in a later chapter

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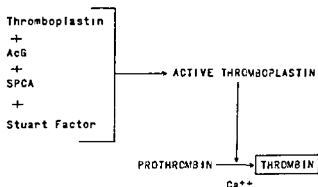


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2 *Clotting Tests**

THE BASIS OF COAGULATION STUDIES lies in the accurate measurement of clotting under controlled in vitro conditions. All information regarding these reactions is inferred from the clotting time or the visible presence of the insoluble fibrin clot. Because of the indirect nature of this information, interpretation of coagulation tests may be complex. Particular attention must be paid to seemingly trivial details in technic, certain conditions (i.e., temperature, ionic strength, and pH) and reagents. Only the tests commonly used as screening procedures in the diagnosis of coagulation defects and those tests which are affected by antithrombotic agents are discussed.[4]

The principal laboratory tests used to determine defects in the three stages of coagulation are listed in TABLE 1. However, it should be noted at this time that two of these tests are of special significance in demonstrating the coagulation defects produced by oral anticoagulants. The first is the thromboplastin generation test, which measures the evolution of intrinsic plasma thromboplastin. Three serum factors, PTC, Stuart, and Hageman, are reduced to less than 10 per cent of normal as measured by this test. Secondly, the one-stage prothrombin test is prolonged by the combined depression of the normal levels of prothrombin and SPCA when oral anticoagulants have been administered.

Heparin, which is administered parenterally, inhibits all coagulation reactions and its effects are measured by the whole blood clotting time, which reflects the over-all coagulability of the blood. Fibrinolysin causes a depression in the normal level of fibrinogen and a rise in the fibrinolytic activity of the blood. This is determined by assay of fibrin produced from a given

* See Addendum for details of the tests.

quantity of the patient's plasma and by the lysis time of the fibrin clot produced respectively

1 *Tourniquet Test* This test measures capillary fragility by applying positive pressure with a blood pressure cuff on the upper arm and inflating the cuff to the midpoint between systolic and diastolic pressure for a period of five minutes After release of the pressure the number of petechiae (ruptured capillaries) are noted on the arm The test is frequently positive in patients who have been given Dicumarol and who have other vitamin

TABLE 1—*Tests for Coagulation Defects*

Tests in Stage 1	*Tests in Stage 2*	*Tests in Stage 3*
Clotting time Thromboplastin generation Clot retraction Prothrombin consumption Tourniquet test	One stage prothrombin Two stage prothrombin Specific one stage tests	Clotting time Fibrinogen

deficiencies such as vitamins C and D The reason for this reaction is related to vessel wall integrity

2 *Platelet Count* The importance of platelets in blood clotting is illustrated by a bleeding diathesis which occurs when the platelet count falls below 50 000 (thrombocytopenia) or rises above 500 000 (thrombocytosis) In either case faulty blood thromboplastin generation results It should be noted that the platelets may be present in normal range (100 000 to 350 000) and bleeding may occur due to qualitatively defective platelets (thrombocytopathia) This condition is demonstrated by an abnormal prothrombin consumption test or a thromboplastin generation test

3 *Bleeding Time* The bleeding time is ascertained normally by making a small puncture in the skin and noting the time of clot formation However excessive tissue damage can result in the release of increased amounts of tissue thromboplastin thus causing a falsely shortened bleeding time Conditions are difficult to standardize with this test therefore quantitative data

cannot be obtained. In general, however, a prolonged bleeding time usually indicates a coagulation anomaly, platelet deficiency or vascular defect.

4. *Clotting Time.* This is a general screening test performed with a venous sample of blood in a Lee-White tube. The time necessary for clot formation is then noted. In general, a prolonged clotting time reflects a disturbance due to a plasma defect of any coagulation factor, the increased titer of an inhibitor or a platelet deficiency. Technical errors (poor vein puncture, air

TABLE 2—*Coagulation Factors Adsorbed from Normal Plasma and Normal Serum with Barium Sulfate*

	Adsorbed Factors	*Nonadsorbed Factors*
Normal Plasma	Prothrombin SPCA PTC Stuart	Fibrinogen AHG AcG PTA Hageman
Normal Serum	Serum AcG PTC SPCA Stuart	PTA Hageman

bubbles and excessive agitation) may nevertheless lead to normal findings even in the presence of a coagulation defect.

5. *Clot Retraction.* A sample of clotted blood in a Lee-White tube begins to pull away from the glass surface within one hour. This is called clot retraction and normally is at its maximum within 24 hours after the sample is taken. Clot retraction is related to the platelets concentration and function.

6. *Preparation of Deficient Plasma.* Certain compounds like chelating agents act specifically to remove some of the factors involved in coagulation. As a group they are called prothrombin adsorbants and the more widely used compounds are barium sulfate and aluminum hydroxide. In the formation of normal serum, fibrinogen, prothrombin and AHG are consumed by the clotting reactions. The factors which can be adsorbed from

normal plasma and normal serum are listed in TABLE 2 Serum and plasma adsorbed with barium sulfate are convenient sources of specific factor deficient solutions which are useful in differentiating coagulation defects when the thromboplastin generation and modified one stage prothrombin tests are performed

7 *Thromboplastin Generation Test* Under certain conditions factors in plasma combine with platelets (or phospholipids) to form a complex with thromboplastic activity This combination is called plasma or intrinsic thromboplastin It differs from tissue thromboplastin in that it is extremely labile due to instability or inhibitors normally present in the blood

The thromboplastin generation test is an assay of this intrinsic thromboplastin and is performed by doing serial one stage prothrombin determinations at one minute intervals A thromboplastin incubation mixture consisting of barium sulfate adsorbed plasma serum platelets and calcium is used The plasma is prothrombin free but contains AHG the serum supplies PTA PTC Stuart factor Hageman factor AcG and SPCA [3] the platelets (or phospholipids) and calcium are the reagents necessary for the formation of thromboplastin

This test demonstrates that patients with hemophilia fail to generate thromboplastin normally By using either normal plasma or normal serum one can determine if the faulty generation of thromboplastin is due to a defect in either plasma or serum Classic hemophilia is due to defective plasma (i e AHG) Only rarely are the platelets thrombocytopathic This is determined by comparing platelets from a normal subject with those of the patient Defects in serum are recognized by the mixture of various serums either congenitally defective or made so with barium sulfate in a specific factor and noting if there is mutual correction of the two serums This type of correction indicates that the factor deficient in one serum is present in the other thus the defects in the two serums are different If the two serums do not correct each other the factor deficient in both are similar For example PTC deficient serum will correct serum deficient PTA Stuart factor Hageman factor and AHG but will not

correct any serum deficient in PTC Studies from this test have *given further proof that hemophilia is a heterogenic group of diseases*

An effect produced by Dicumarol and related drugs is a depression of PTC Stuart factor and Hageman factor which are not measured by the one stage prothrombin time This fact may be another explanation for the frequent clinical observations of purpura and bleeding in patients on long term anticoagulant therapy when the prothrombin time is within the therapeutic range

8 *Prothrombin Consumption* The clotting of normal blood results in the formation of plasma thromboplastin with the subsequent conversion of prothrombin to thrombin The latter is partially absorbed by the fibrin clot in the final stage of coagulation what remains is neutralized in the serum by antithrombin When there is a defect in the formation of intrinsic thromboplastin the prothrombin is not fully utilized and remains in the serum If a one stage prothrombin determination *is done on the serum by adding fibrinogen an estimate of the* prothrombin remaining in the serum can be obtained This test is called prothrombin consumption and can be expressed in percentage of prothrombin used or remaining in the serum

Since the conversion of prothrombin to thrombin is a relatively slow reaction the time after clotting for performance of the prothrombin consumption test is usually one hour This test is useful in indicating defects in the first stage of clotting but is of little value in patients given Dicumarol The reduction in plasma prothrombin resulting from such therapy leaves the titer of serum prothrombin beyond the sensitivity of the test This is true because the prothrombin consumption test is subject to the same technical errors as the one stage prothrombin time

9 *Prothrombin Time* The test known as the one stage prothrombin time is simple reproducible and has worldwide application in clinical medicine This test measures the velocity of fibrin formation following the addition of calcium to a solution

of active tissue thromboplastin and the plasma to be tested The most important variables affecting the test are factors which aid or inhibit the formation of thrombin The conversion of fibrinogen to fibrin in the presence of thrombin is almost instantaneous The time interval from the addition of calcium to the formation of the clot is the prothrombin time (PT) Dependent upon the reagents used and the technician performing the test the PT is usually 12 seconds with normal plasma If the thromboplastin accelerator factors calcium or prothrombin are progressively reduced the PT is increased

A normal prothrombin time generally means that all factors other than those necessary for the formation of thromboplastin are present in normal concentrations However a prolonged prothrombin time is difficult to interpret since the result can mean a reduction from the normal level of one or several of the following prothrombin AcG SPCA Stuart factor or fibrinogen In addition an abnormal prothrombin time may mean an increase in either the level of naturally occurring or acquired inhibitors to any of these factors or an inhibitor to the active agent produced from the interaction of these factors Dicumarol produces depression of prothrombin SPCA and the Stuart factor Therefore a prolonged prothrombin time in patients on this therapy represents a combined defect produced by the decreased level of these proteins Heparin inhibits all coagulation reactions thereby giving a prolonged prothrombin time

The one stage prothrombin test can be modified by the addition of prothrombin free plasma This modification will reflect the prothrombin level more accurately since everything except the factor tested for will then be present in the reaction mixture

10 *One Stage AcG Determination* Specific determination of AcG is done by adding aged normal plasma to the plasma to be tested and then performing a one stage prothrombin test Normal plasma aged in the refrigerator for 14 days is deficient in AcG because of its lability Therefore all the factors necessary for clotting are present in the incubation mixture except AcG the factor being tested in the sample plasma

11. *SPCA Determination.* The assay of the level of SPCA is determined with a one stage prothrombin test modified by adding an SPCA free plasma to the test plasma. A variable in this test is the amount of SPCA contaminating the tissue thromboplastin used. This is especially true in patients given Dicumarol when the level of SPCA is depressed to 10 per cent or less of normal.

12. *Two Stage Prothrombin Determination.* Specific assay of prothrombin is accomplished with the two stage prothrombin test. The basis for this test is not velocity but rather the measurement of the amount of thrombin generated. It is performed by activating plasma with optimal amounts of calcium, thromboplastin and the accelerators (AcG, SPCA and Stuart factor). Thus the plasma prothrombin is activated to thrombin and serial aliquots are tested with purified fibrinogen. Applying the principle that a shorter clotting time means more thrombin, one can use this technic to obtain a semi quantitative measurement of the thrombin present in the sample. One unit of thrombin is defined as the amount of thrombin which will clot 1 cc of fibrinogen in 15 seconds at 29 C. One unit of thrombin is equal to one unit of prothrombin. This technic has not been used generally as a guide in the control of anticoagulant therapy because of the technical difficulty involved and also because the test measures only one factor depressed by oral anticoagulants.

13. *Fibrinogen Determination.* Fibrinogen is determined by adding thrombin to a measured quantity of citrated plasma and winding the clot on a glass stirring rod. The fibrin is digested from the glass rod with biuret solution and the amount of protein is determined by noting the color change in the biuret solution with a colorimeter and by comparing the reading to a standardized curve.

14. *Fibrinolysin Determination.* The fibrinolytic activity in serum is determined by noting the lysis time of a standard fibrin clot with a measured amount of serum.

3 *Antithrombotic Reactions*

AS MENTIONED EARLIER the body has natural defense mechanisms to prevent limit and dissolve intravascular clotting This is accomplished by inhibitors that suppress the princpal coagulation reactions and by fibrinolysin which dissolves the fibrin clot In addition a hypocoagulable state may be induced by anticoagulants which are of two main types (1) those (such as heparin) which prolong the over all clotting time and (2) those (such as Dicumarol) which indirectly suppress the synthesis of certain coagulation factors Fibrinolytic agents such as streptokinase [18 0] trypsin [103] and fibrinolysin [44–46] have been introduced more recently into clinical medicine The mechanisms of these natural and therapeutic defenses against intravascular clotting are detailed in this chapter their clinical applications are discussed later

Naturally Occurring Anticoagulants

1 *Inhibition of Stage I* Specific antibodies may develop in plasma which inhibit AHG PTC or AcC respectively [73 74] Antibodies have also been described which destroy the platelets thereby leading to a hemorrhagic diathesis In addition plasma contains an inhibitor of thromboplastin [188 191] but it is still uncertain whether this material differs from heparin

2 *Inhibitors of Stage II* A specific antibody inhibitor of AcG exists but specific inhibitors of SPCA or the Stuart factor have not been reported However in normal serum a factor (or factors) called antithrombin [31 11] is present which progressively inactivates thrombin forming a complex called metathrombin in the older literature This is partially reversed in vitro by the addition of acids alkalies or chloroform Antithrombin is the body s defense against circulating thrombin The action of antithrombin is synergistic with heparin

3 *Inhibitors of Stage III* The natural defense against fibrin is the lysis of the insoluble clot into soluble fragments by fibrinolysin (plasmin) This enzyme is normally present in the blood as an inactive precursor (profibrinolysin plasminogen) which forms fibrinolysin under the influence of an activator This activator is produced from a proactivator present in plasma in combination with a factor derived from damaged tissues foreign bacterial products or idiopathically Presumably the activator is also formed by the combination of the proactivator with a tissue factor It then enzymatically converts profibrinolysin to its active form fibrinolysin The latter acts as a proteolytic enzyme that splits both fibrinogen and fibrin in vivo and in vitro into protein fragments that do not clot with the addition of thrombin Clinical conditions exist in which a hemorrhagic diathesis is associated with an increasing circulating titer of fibrinolysin[190]

Since fibrinolysin is not only a physiologic restorer of hemostasis but also a therapeutic agent it is discussed further in the concluding section of this chapter

Therapeutic Antithrombotic Agents

The problem regarding the therapeutic approach to intravascular clotting involves two methods (1) the indirect prophylactic prevention of coagulation and (2) direct lysis of the clot Heparin and Dicumarol are examples of agents representing the first method and fibrinolysin the second method

1 *Heparin* The mode of action of heparin is inhibitory in all three major clotting reactions and its effect is measured by the prolongation of the clotting time Heparin plus a postulated cofactor in the plasma forms an immediate antithrombin which inhibits fibrin formation particularly Observation of the reactions involving heparin suggests that it combines with proteins altering their electrochemical and colloidal properties

Heparin must be administered parenterally and the preferred route is intravenous Its action is fast but its potency is rapidly

destroyed This is the major disadvantage to its therapeutic use since repeated injections are necessary Clinical application of heparin is thus limited to the initiation of anticoagulant therapy At present there are no adequate synthetic substitutes for heparin although several agents have been investigated

2 *Oral Anticoagulants* Oral anticoagulants such as Dicumarol and related drugs inhibit the synthesis of prothrombin SPCA PTC Hageman and the Stuart factors [141 1] which are produced by the liver in the presence of vitamin K Apparently Dicumarol functions is a competitive inhibitor of the utilization of vitamin K by the liver A number of drugs functioning similarly to Dicumarol are used clinically These drugs may be divided into two groups coumarins (Dicumarol Sintrom Tromexan Marcumar Coumadin and Cumopyran) and (2) indandione derivatives (Hedulin Dipaxin and Miradon) The major differences in these drugs are in the speed and duration of action these are dependent upon the rate of absorption into the blood Those which are rapidly absorbed are quick to act but of short duration The converse is also true as is clearly illustrated by the one stage prothrombin test

Dicumarol is eliminated chiefly by the kidneys therefore renal disease can cause a more sensitive response to anticoagulants Still other factors influencing the response to Dicumarol are both the vitamin K intake and the amount of vitamin K produced by intestinal bacteria Antibiotics which destroy the intestinal flora are synergistic with oral anticoagulants Aspirin also has a mild Dicumarol like action and acts synergistically when administered with anticoagulants An example of this effect is shown in FIGURE 1

Recent studies have demonstrated a fall in prothrombin SPCA PTC and fibrinogen in patients after hepatic lobectomy for neoplastic diseases Liver disease when severe causes a reduced level of the various coagulation factors and is associated clinically with an increased sensitivity to Dicumarol

The coagulation defects produced by Dicumarol are decreased levels of SPCA prothrombin PTC Stuart and Hageman fac

tors in that order The rapidity in the fall of these various factors is related to the dose of the anticoagulant and the turn over rates of the coagulation factors The action of Dicumarol and related drugs may be reversed by the intravenous administration of vitamin K_1 [3] The effects are noted on the one stage prothrombin time within two to six hours Vitamin K deficiency of various types [11] (i e steatorrhea and malnutrition) is asso

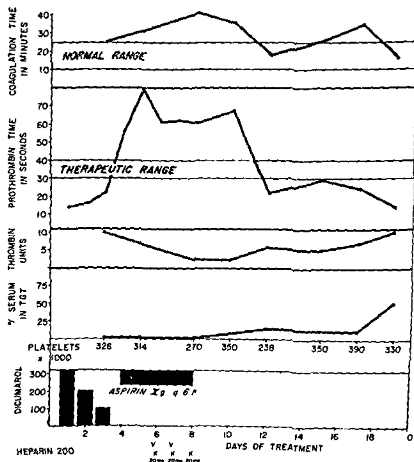


Fig 1—The prolongation of Dicumarol action by aspirin in a patient with acute myocardial infarction It should be noted that intravenously administered vitamin K_1 had little effect on the prothrombin time until the aspirin was stopped

ciated with reduction in SPCA prothrombin PTC Stuart and Hageman factors

3 *Thrombolytic Agents* Agents which promote the dissolution of the fibrin clot have been investigated extensively in the last few years and are classified under the general term of fibrinolytic agents They act directly on the formed clot and as such present a direct approach to intravascular clotting The first agent with fibrinolytic activity studied was streptokinase This material was found in the filtrates of beta hemolytic streptococcic cultures by Tillett and Garner in 1933 [44] Streptokinase combines with the proactivator in the plasma [39] to form an enzyme which activates profibrinolysin which subsequently acts on both fibrinogen and fibrin (See DIAGRAM 4 for this reaction)

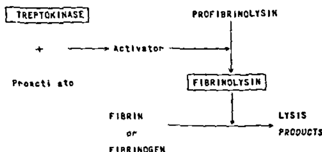


DIAGRAM 4—Fibrinolytic reactions

The activity of streptokinase therefore depends upon the concentration of several inhibitors Varying concentrations of circulating antistreptokinase and antifibrinolysin result in a markedly individual response to an injection of streptokinase [24] Another problem with this therapy is that once an active circulating fibrinolytic system is induced there is no specific method available for reversing the active enzyme Concentrates of fibrinogen can be administered but these are usually contaminated with profibrinolysin and proactivator which tend to propel the reaction further and thus increase the fibrinolytic activity In addition to this even the purest preparations of

streptokinase are associated with systemic reactions which preclude its general clinical use at the present time

Trypsin was explored as a thrombolytic agent in patients but because of the protein nature of its activity and the presence of antitrypsin factors in the plasma [73] large parenteral dosages had to be administered This has proved to be clinically inadvisable because of the shock syndrome associated with intravenous injections [40]

More recently with the advent of technical advances in the purification of human fibrinolysin [8] the use of intravenous fibrinolysin has been tested in animals and man [14, 34, 35, 41, 46] An advantage of this enzyme is its specific proteolytic action on fibrinogen and fibrin The level of fibrinolytic activity induced is variable depending on the level of antifibrinolysin in the plasma but levels of fibrinolysin can be injected to overcome this variation The fibrinolytic activity can either be maintained by continuous infusion or reduced by slowing or stopping the infusion Preliminary clinical reports in man have shown that fibrinolysin is effective and practical [150, 30] Toxic reactions [3] indicate cautious administration of this agent until further clinical evaluation is obtained

In combination with anticoagulants fibrinolysin seemingly offers an ideal approach to the treatment of intravascular thrombosis The tests available for controlling fibrinolysin therapy are the determination of fibrinogen and the lysis time of standard fibrin clots

4 *Clinical Use of Antithrombotic Agents*

THE AIM OF ANTITHROMBOTIC THERAPY is to reverse the occlusion of a vessel by disintegration of the clot and the prevention of subsequent thrombotic formation. Fibrinolysin will dissolve an existing fibrin clot; anticoagulants will inhibit further clotting.[43, 3, 5, 69, 70] The limiting factor in the use of this therapy is the risk of hemorrhage. Fibrinolysin has not been used extensively and caution is indicated until further evaluation has been performed. In this chapter an outline of the usual effective dosages of anticoagulants and a discussion of the treatment of hemorrhage will be presented.

Preparation of the Patient

When anticoagulant therapy is to be used, the patients should be selected carefully with regard to personality and living conditions which may prevent close cooperation between patient and physician. The patient should fully understand the purpose and possible dangers of anticoagulant therapy and, in addition, should be given written instructions and dosage schedules. As a precaution against unexpected bleeding, the patient should be provided with vitamin K_1 tablets and be instructed to carry them with him at all times.

Heparin

Heparin is a mucopolysaccharide which is practically nontoxic. The preferred route of administration is intravenous. Oral heparin is inactive,[11] and the rate of absorption is quite variable when the drug is used intramuscularly or subcutaneously;* therefore control is difficult. Heparin sodium is avail

* Subcutaneous heparin administered with a [illegible] needle has been used successfully as a practical method at the Miami Heart Institute.

able in sterile aqueous solutions in strengths that contain 1 000 *5 000 10 000* or *20 000 U S P* units in *1 ml* The usual initial dose is 50 mg (5 000 units) If the clotting time which is done four hours later is less than twice the control clotting time the second dose should be increased 50 per cent If the clotting time is triple the control clotting time further injection of heparin should be withheld until the clotting time returns to the desired range

Rare instances of hypersensitivity urticaria asthma and allergic rhinitis have occurred from the administration of heparin Contraindications to the use of heparin are the following bleeding tendency or purpura subacute bacterial endocarditis ulcerative lesions of the gastrointestinal tract intracranial hemorrhage threatened abortion and hypersensitivity to heparin

Should it be necessary to terminate heparin therapy rapidly or should bleeding develop the activity of heparin can be quickly neutralized by slowly administering 50 mg of protamine sulfate intravenously In addition whole blood or plasma may be given if the bleeding becomes life threatening Heparin therapy is usually maintained for 24 to 48 hours until the oral anticoagulants have become effective as demonstrated by prolongation of the prothrombin time namely two and one half times longer than the control prothrombin time in seconds

Oral Anticoagulants

Dicumarol was the first oral anticoagulant[1, 6, 91] to be used clinically and has an indirect action on the clotting factors by suppressing the synthesis of prothrombin SPCA PTC Stuart and Hageman factors Because of the indirect nature of its activity 24 to 48 hours or longer are required before the circulating level of these various factors is depressed to inhibit coagulation effectively FIGURE 2 illustrates the effect of Dicumarol on the various coagulation factors It antagonizes the action of vitamin K in the liver which is a necessary vitamin for the formation of clotting proteins

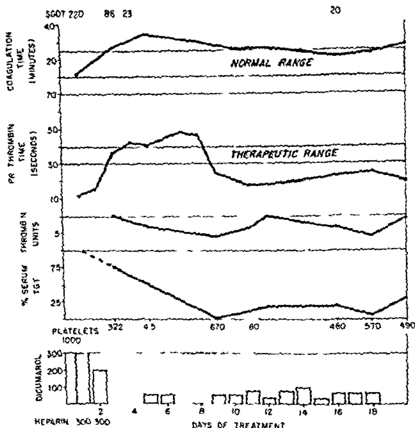


Fig 9—Rate of depression in the various coagulation tests from Dicumarol therapy in a patient with myocardial infarction The prothrombin time is 3 seconds on the third day of treatment and thromboplastin generation was nearly zero on the ninth day of treatment

The newer oral anticoagulants function like Dicumarol The effectiveness of the drugs is directly related to the blood levels thus factors of absorption from the gut and excretion via the kidney are important considerations in therapy Diarrhea vomiting aspirin antibiotics diuretics and the vitamin K content in the diet[101] are some of the various factors altering

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a more protracted effect It is available in intravenous form which speeds up its initial effects to within 24 hours thereby reducing the need for heparin therapy except for the first day The intravenous dose is 75 mg and maintenance is usually obtained with 10 mg orally each day This drug has proven to be a useful anticoagulant by several different observers [17]

Cumopyran (methopyranorin) is about three times as potent as Dicumarol and has a slightly more rapid onset of action than Dicumarol and a prolonged therapeutic effect

Marcumar (phenprocoumon) [3, 189] is a more potent drug than Dicumarol and becomes effective within 36 hours and its efficacy lasts from two to three days Several reports have indicated that Marcumar is an effective anticoagulant and it is interesting to note that this drug will depress the serum thromboplastic factors [37] despite a report suggesting no depression of PTC [11, 114]

Sintrom (acenocoumarin) is also more potent than Dicumarol and becomes effective within 36 hours with a duration of action lasting 48 to 60 hours This drug has been used successfully in many patients and the dosage necessary is relatively constant

Hedulin or Danilone (2 phenyl 1 3 indandione) [78] is one of the indandione derivatives The drug produces a depression of prothrombin time within 48 hours and the effect of the initial dosage is dissipated within another 48 hours There are probably fewer bleeding episodes with this drug However a number of drawbacks to its use are the frequent resistance to its action and discoloration of the urine which may be confused with bleeding There have also been a few reports of bone marrow depression urticaria and dermatitis

Dipaxin (2 diphenylacetyl 1 3 indandione) is more potent than Hedulin and also has a more prolonged effect However like all the indandione derivatives it imparts an orange color to the urine which can cause confusion in regard to urinary bleeding

Miradon (anisindone or 2 p anisyl indandione 1 3) becomes effective within 36 to 48 hours and maintains a fairly stable

sensitivity to Dicumarol. It has been given to patients for many years without any evidence of toxic effects. Bleeding occurs in a small percentage of patients and will be discussed later in the chapter. Some patients have toxic manifestations such as nausea, vomiting and diarrhea even with therapeutic dosages.

Contraindications to Dicumarol are the same as heparin. However, additional contraindications are recent operations on the brain or spinal cord, regional anesthesia and lumbar block, vitamin K deficiency and severe hepatic[7] or renal disease.

TABLE 3.—*Average Dosage of Oral Anticoagulants*

Anticoagulant	*During First 48 Hrs (mg.)*	*Usual Maintenance Dose (mg.)*
Dicumarol	400–500	5
Tromexan	1800–2400	4 0
Coumadin	50–100	10
Cumopyran	100–200	25
Marcumar	21–30	3
Sintrom	18–24	4
Hedulin	250–400	100
Dipaxin	24–30	3
Miradon	600–900	75

The drug should be used with caution in patients with severe hypertension, active pulmonary tuberculosis and general debilitating diseases.

Five other coumarin derivatives and three indandione preparations are available and have been used clinically as effective oral anticoagulants.[55, 6, 66, 71, 1] The usual dosages of these various oral anticoagulants are summarized in TABLE 3.

Tromexan (ethyl biscoumacetate)[114] is less potent than Dicumarol but has the advantage of a more rapid therapeutic effect (24 to 36 hours). However, its disadvantages are a rapid dissipation of effect, variability in maintenance dosage and frequent episodes of "escape" from therapeutic effects.

Coumadin (sodium warfarin)[1] is a water soluble coumarin which is about five times more potent than Dicumarol but has

a more protracted effect. It is available in intravenous form which speeds up its initial effects to within 24 hours, thereby reducing the need for heparin therapy except for the first day. The intravenous dose is 75 mg, and maintenance is usually obtained with 10 mg orally each day. This drug has proven to be a useful anticoagulant by several different observers.[17]

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do occur and the use of anticoagulants carries a certain risk. The estimated mortality rate is 0.2 per cent.[60] Excessively prolonged prothrombin times are generally the result of improper use of the drugs, although bleeding does occur in the presence of "therapeutic" prothrombin levels. The latter is especially true in patients on long term therapy. The incidence of hemorrhage appears to be similar for the various coumarin drugs but it is somewhat less when the indandione derivatives are used. The most frequent type of hemorrhage is gross or microscopic hem-

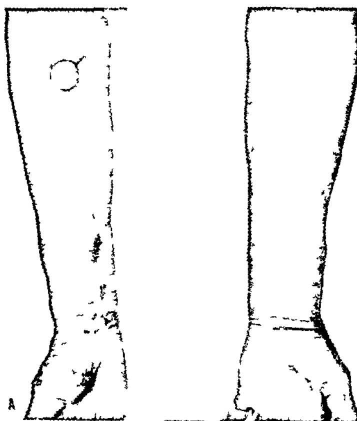


Fig. 3A and B—Two common manifestations from anticoagulant therapy.
Fig. 3A (above)—Patient with petechiae from anticoagulant therapy.

anticoagulant effect However it offers no special advantage over the previously mentioned drugs

Management of Oral Anticoagulant Therapy

When the need for anticoagulant therapy has been established and it can be determined that the patient has no contraindication to the use of anticoagulants therapy should be started immediately Heparin is administered in combination with an oral anticoagulant The heparin is continued until the prothrombin time has reached a level two and one half times the control prothrombin in seconds This is generally 25 to 30 seconds The blood for the prothrombin determination should not be obtained within four hours after the heparin administration Heparin causes prolongation in the prothrombin time determination Anticoagulant therapy is maintained by a daily dose given at the same time each day The daily dosage required to maintain a therapeutic prothrombin time is fairly constant in a patient If the prothrombin time is greater than 40 seconds the drug is reduced or withheld until the prothrombin time returns to the range of 25 to 40 seconds After the patient s response to a particular drug has been determined prothrombin times can be done at progressively longer intervals of time namely weekly or at two week intervals Some authors have reported on the use of an intermittent dosage schedule but this method has the disadvantage of wide swings in prothrombin times which means long periods without adequate anticoagulant activity

Complications of Anticoagulant Therapy

The most important complication of anticoagulant therapy is hemorrhage [16] Hemorrhage is rarely a problem in patients treated for acute episodes such as myocardial infarction but about nine per cent of the patients have significant hemorrhage In patients treated with long term anticoagulants the incidence of hemorrhage is 20 per cent or higher if treatment is continued for several years The bleeding is rarely serious but fatalities

intravenously.[3] This will bring the prothrombin time to a safe level within four to six hours. If necessary, vitamin K_1 can be repeated in several hours although a second dose is seldom required. The prothrombin time can be corrected quickly by transfusions of fresh blood or plasma when dangerous bleeding ensues.

In the treatment of patients with acute myocardial infarction

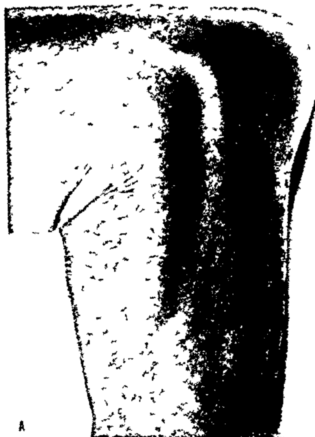


Fig. 4A and B—Two cases of bleeding as manifestation of anticoagulant therapy. Fig. 4A (at left)—Hemorrhage into elbow joint.

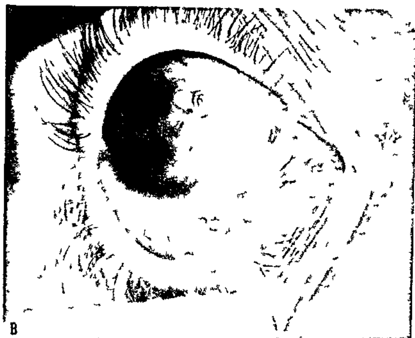


Fig 3B—Patient with subconjunctival hemorrhage from anticoagulant therapy

turia and petechiae Ecchymosis hematemesis or melena and epistaxis occur less frequently and in women menorrhagia is a common occurrence (See FIGURES 3 and 4)

Gross hemorrhage following heparin is generally due to some local pathologic condition When this occurs transfusion of 500 cc of fresh whole blood or plasma should be given immediately It may be necessary to continue or repeat the transfusion if bleeding does not stop Occasionally intravenous protamine sulfate or toluidine blue are given However this is seldom necessary when heparin is administered intravenously since its activity is quickly dissipated in four to six hours Should the bleeding be due to prolonged prothrombin time the patient can be treated by reducing the dose of the anticoagulant or by administering 5 to 50 mg of vitamin K_1 (Mephyton*) orally or

present recurrent chest pain without electrocardiographic evidence of extension of the recent myocardial infarction persistent pericardial friction rub sudden shock hepatomegaly distention of neck veins sudden anemia or x ray evidence of pericardial effusion The presence of hemopericardium is an indication for the stoppage of anticoagulant therapy Several reports have indicated an increased incidence of myocardial rupture in patients treated with anticoagulants as compared to untreated patients with acute myocardial infarction [5] However an analysis of autopsy data reveals that the incidence of myocardial rupture is similar for a group of patients over a five year period prior to anticoagulant therapy and for a comparable group over a five year period during anticoagulant therapy Although myocardial rupture was more common in patients treated with anticoagulants no significant over all increase in the incidence of myocardial rupture was found since the advent of anticoagulant therapy [170]

Cerebral hemorrhage is a serious complication of anticoagulant therapy and is an indication for its discontinuance The presence of severe hypertension is considered to be an important factor predisposing patients to cerebral hemorrhage from anticoagulant therapy [179]

In patients on long term anticoagulant therapy there are frequent episodes of bruising and purpura without gross bleeding This relatively frequent occurrence has led some investigators to suggest that perhaps the same degree of clinical effectiveness might be obtained with less depression of the coagulation factors The danger of not maintaining a sufficiently prolonged prothrombin time is illustrated in TABLE 4

The patient described in TABLE 4 had been on Dicumarol therapy for two years following a myocardial infarction without any evidence of recurrent thromboembolism During a six month period his prothrombin time fell to 17 seconds or below for *two separate two week periods During the second period* of two weeks when his prothrombin time was 17 seconds he developed a cerebral vascular accident which was clinically

there are two complications that deserve special attention hemo pericardium and myocardial rupture Hemopericardium should be suspected when one or more of the following findings are

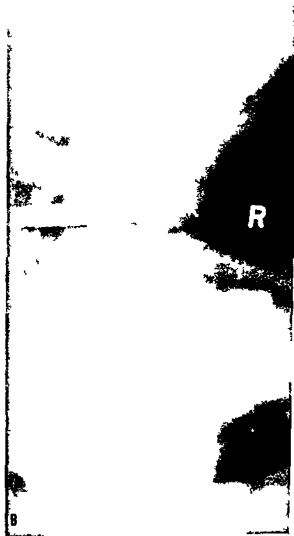


Fig 4B—Spinal cord block secondary to hemorrhage and clot formation (Reproduction of the myelogram by permi si n of Freder ck A Loppe M D Coral Gables Fla)

therapeutic range represents a problem for further investigation. The importance of this problem is illustrated by several case reports of unsuspected cerebral hemorrhage. TABLE 5 illustrates the effect of vitamin K_1 on bleeding in two patients given Dicumarol over a long period of time. Recent studies have shown that there is a serum defect in these patients which can be demonstrated by the thromboplastin generation test but

TABLE 5—*Effect of Vitamin K_1 on Bleeding during Dicumarol Therapy*

Patient	Date	Clotting Time (min)	Prothrombin Time (%)	AC (%)	SPCA (sec)	Serum Thromboplastic Factor (%)	Remarks
M. F.	10/8	3[illegible]	[illegible]0	[illegible]0	[illegible]	10	Bleeding;
	11	—	8	—	—	—	given 5 mg
	12/8	0	3	4	[illegible]	0	vitamin K_1
	1	14	14	80	[illegible]	100	orally
	12	14	1	[illegible]0	14	100	on 1/8
J. D.		56		0	[illegible]	0	Bleeding;
	6/11	3[illegible]		60	1[illegible]	10	given 10 mg
	6/12	3	[illegible]	80	13	[illegible]0	vitamin K_1 [illegible]

which is not measured by the prothrombin time. Several investigators have reported that this serum factor is similar to PTC.[141, 155] Our studies have shown that the defect is PTC, Stuart and Hageman factor depression. This is shown in FIGURE 5. The defect is produced by all the oral anticoagulants. This serum defect cannot explain all the bleeding episodes because most patients on long-term anticoagulant therapy have a marked depression of the serum thromboplastic factors (FIGURE 6); however, fewer than 10 per cent of the patients have clinical evidence of any type of bleeding. Fluctuation of the different factors is presented in FIGURE 7.

Sise et al. have recently reported that the important factor in recurrent thrombosis or hemorrhage is the level of circulating

diagnosed as thrombosis or embolism Other patients occasionally develop thromboembolic complications when their prothrombin times are in therapeutic ranges but usually there is some underlying complication such as a hidden neoplasm Therefore if effective anticoagulant therapy is to be maintained

TABLE 4—*Recurrent Thromboembolism in a Patient on Dicumarol*

Date	*Prothrombin Time (sec)*	*Average Daily Dose (mg)*	*Remarks*
3/18	[illegible]0	[illegible]	
3/2[illegible]	26	[illegible]	
4/1	[illegible]3	[illegible]8	
4/8	[illegible]	[illegible]	
4/1[illegible]	[illegible]	[illegible]	
4/2[illegible]		2	
[illegible]/ 9	[illegible]2	[illegible]	
13	13		
[illegible]/20	16 [illegible]	8	
[illegible]/27	19	[illegible]	
[illegible] 3	[illegible]1	[illegible]	
6/10	31	8	
6/17	3[illegible]	6[illegible]	
6/[illegible]4	[illegible]4	68	
[illegible]/1	1	6[illegible]	
[illegible]/8	1	[illegible]	Cerebral vascular
[illegible]	[illegible]	8[illegible]	accident (embolism)
[illegible]/29	[illegible]4	8[illegible]	
[illegible]	3	4	
8/1[illegible]	[illegible]3	[illegible]3	

Control prothrombin time—1[illegible] seconds

the patient's prothrombin time must be held in the therapeutic range of 25 to 10 seconds (control prothrombin time is twelve seconds)

Bleeding from Long Term Anticoagulants

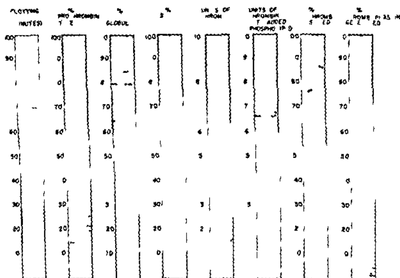
When the prothrombin time is prolonged excessively bleeding is easily explained on the basis of the greatly delayed clotting[59, 65, 181, [illegible]9] However bleeding in patients on long term anticoagulant therapy in whom the prothrombin time is in the

TABLE 6—*Coagulation D fects from Long Term Dicumarol*

Tests for Coagulation D fects	*Hist ry of Nonbleed in—(3 patie ts)*	*History of Bleeding—(49 patients)*
Prothrombin time (%) (one stage meth d)	1	20
SPCA ( )	10	8
Prothrombin ( ) (two stage method)	50	39
Serum defect in thrombo plas tin generation test ( )	(	10

tients with no history of bleeding as compared to 19 patients with past episodes of frequent bleeding A summary of these data is shown in TABLE 6

Studies by Verstraete et al have shown that one factor commonly overlooked in the performance of the one stage prothrombin test is the difference in SPCA like activity in various



Fi —Effects of long term anticoagulant therapy in the various coagulation factors The shaded areas represent the normal range for the various tests The lots repres nt the actual value obtained in the various tests From Bosles et al Blood 14 P1 19 9 rep odu ed by permi n of the publisher

prothrombin In a series of 86 patients given Dicumarol for a period of one to 10 years we have been unable to demonstrate any significant difference in the coagulation defects in 37 pa

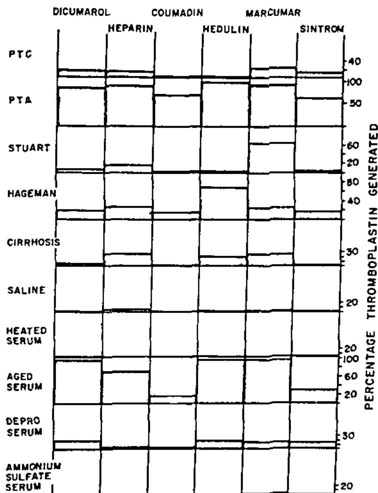


Fig 5—Percentage of thromboplastin generated from a mixture of 50 per cent serum from patients treated with various long term anticoagulants with 50 per cent serum congenitally deficient in a specific factor sera from a patient with severe liver disease and normal serum treated with physical or chemical agents It will be noted that the defects in thromboplastin generation caused by the various anticoagulants are similar namely depression in the normal levels of PTC Stuart and Hageman factors

Frequently prothrombin times done with thromboplastin containing no SPCA like activity are prolonged beyond 40 seconds whereas the test is within the therapeutic range and below 40 seconds when performed with other thromboplastin preparations which contain trace SPCA like activity

The modified one stage prothrombin test of Owren Ware Stragnell measures prothrombin primarily [1 31] as does also

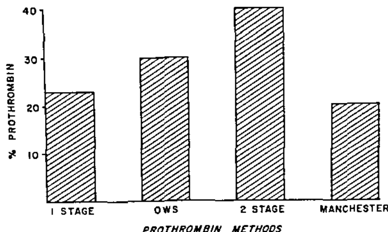


FIG 8—The various prothrombin levels in patients on long term anticoagulant therapy (100 patients) The amount of prothrombin assayed by the two stage method is almost twice the amount assayed by the Quick one stage prothrombin time

the two stage method of Ware and Seegers However the one stage test measures the relative depression of prothrombin SPCA and Stuart factor in patients on anticoagulant therapy Another method which is not used generally is the simple bedside test of Manchester [133] Comparison of these methods in patients on long term anticoagulants is shown in FIGURE 8

Discontinuance of Anticoagulant Therapy

One major disadvantage of anticoagulant therapy is the danger of interruption for any reason such as hemorrhage

thromboplastins We have confirmed these findings but the clinical importance of this factor in bleeding when prothrombin times are in the therapeutic level has not been determined

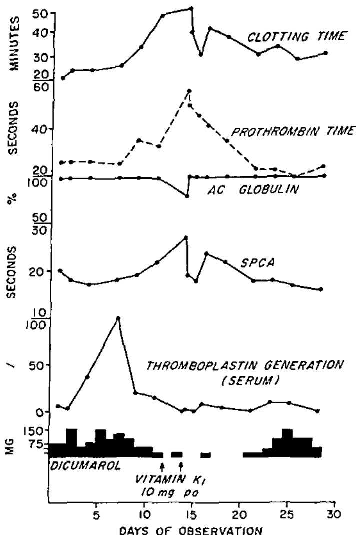


Fig 7—The relative independence of the various coagulation tests in a patient on long term Dicumarol therapy Of particular interest is the dissociation between the prothrombin time and thromboplastin generation curve From Bowles et al Blood 14 181 1959 reproduced by permission of the publisher

but a recent report of prolonged bleeding from dental extraction [83] has indicated the danger of such procedures

Therefore in patients in whom dental or surgical procedures are planned anticoagulant therapy should be discontinued three days prior to the operation After the procedure reinstitution of anticoagulants should be carried out with heparin and oral anticoagulants If discontinuation of anticoagulants is planned the therapy should be reduced gradually over a period of several weeks in order to reduce the danger of the rebound phenomenon

operation or dental extraction The discontinuance of therapy not only restores the risk of thromboembolism but there is also a rebound phenomenon with a return of the coagulation factor back to normal or above normal levels for a temporary period as sugggested in several case reports Carter et al[4] have

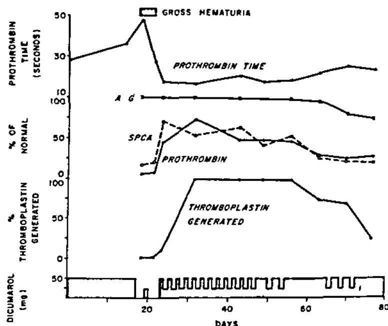


FIG 9—The relative levels of the various coagulation factors in a patient bleeding from long term Dicumarol therapy The rapid (rebound) of SPCA and prothrombin (two stage method) are in marked contrast to the sluggish return of the thromboplastin generation test to normal

recently presented statistical data that indicate that the tendency toward recurrent thromboembolism is greatest during a six week period following such an episode or the discontinuation of anticoagulant therapy in patients without a recent thromboembolic attack (See FIGURE 9)

Several reports have indicated that dental extraction can be carried out while a patient is undergoing anticoagulant therapy

patients treated conservatively compared to 0 15 per cent in 900 patients treated with either heparin or Dicumarol In the prophylactic use of Dicumarol postoperatively the incidence of fatal pulmonary embolism was reduced from 6 to 0 per cent in a total of 1 332 patients These studies [16 5 93] have convincingly established the value of anticoagulant therapy in these conditions

Acute Myocardial Infarction

Anticoagulant therapy for acute myocardial infarction has been the subject of much controversy As early as 1938 animal

TABLE [illegible]—*Effect of Anticoagulant Therapy on the Mortality from Acute Myocardial Infarction*

Author	Control Group		Treated Group	
	Patients (No)	Mortality (%)	Patients (No)	Mortality (%)
[illegible] (1946)[184]	60	1	0	4
Nichol and Page (194[illegible])[16]	—	—	44	18
[illegible]rker and Barker (194[illegible])[1]	100	40	0	10
Peters (1948) [84]	8[illegible]	2[illegible]	110	11
[illegible]riesman and Marcus (1948) [illegible]0	100	3	[illegible]	9
Ch[illegible]k (1948)[8]	44	45	41	[illegible]
Wright et al (1948) [illegible]	368	4	43	15
[illegible]e[illegible]ton and Taylor (1950)*	4	[illegible]	4	[illegible]0
Holton (1950)[10]	[illegible]6	36	1[illegible]4	2[illegible]
Z[illegible]uff and Field (19[illegible]0) [illegible]8	100	40	80	[illegible]
Tulloch and G[illegible]lchr[illegible]t (1950) [illegible]	84	40	[illegible]0	[illegible]3
S[illegible]hill[illegible]g (19[illegible]0)	60	40	60	1
Tulloch and G[illegible]lch[illegible]st (195[illegible]) [illegible]0	161	43	1[illegible]	21
R[illegible]hkoff (19[illegible])	14	[illegible]	14[illegible]	1[illegible]
Ker[illegible]n (1953)	160	[illegible]9	32	18
Furman (1953)[9]	211	3	100	18
Schnur (19[illegible]3)	1 350	30†	81	10
Beaumont (19[illegible]3)	96	3	1	10
L[illegible]ud[illegible]n (1953) [illegible]8	1	41	7[illegible]	[illegible]
M[illegible]l[illegible]e[illegible]ter (19[illegible]4) [illegible]3	1[illegible]0	2[illegible]	1[illegible]0	1[illegible]
W[illegible]ght AHA Study (19[illegible]4)	41	[illegible]3	89	16

* Hosp[illegible]al [illegible]rd.
† App[illegible]m[illegible]t
‡ Qu[illegible]ted by Z[illegible]l[illegible]ff [illegible]nd Field [illegible]

5 *Present Status of Anticoagulant Therapy*

DURING THE PAST 15 years anticoagulant therapy has been used for various thrombotic and thromboembolic disorders.[4, 40, 4, 7, 11, 10, 103, 1, 14, 4, 38, 4] Despite numerous investigations on the effectiveness of anticoagulant therapy its use is still highly controversial. The real value of anticoagulant therapy remains unknown because there are no conclusive therapeutic studies. *Statistical data are available on patients with acute* thrombotic and recurrent thromboembolic episodes. Admittedly this type of information is not conclusive but it does give some indication of the general clinical effects of anticoagulant therapy.[64, 84, 88, 103, 110, 118, 163, 187, 40, 73]

At present there is no satisfactory laboratory aid to predict which patients when subjected to operation or bed rest will develop thromboembolic complications. The following patients have been found on the basis of clinical observations to be benefited by anticoagulant therapy: (1) All patients who have a past history of any thromboembolic or occlusive arterial disease, (2) all patients following acute coronary occlusion, (3) patients over 50 years of age (without any specific contraindication) and (4) *patients in congestive heart failure or auricular* fibrillation, obese, debilitated, polycythemic or anemic.

In this chapter an analysis will be made of the current status of anticoagulant therapy in various thrombotic disorders.

Thrombophlebitis and Pulmonary Embolism

Wright[3] has reported on the effectiveness of anticoagulant therapy in thrombophlebitis and pulmonary embolism. Total embolism was reduced from 5.7 to 0 per cent in the former and from 18.3 to 0.3 per cent in the latter. Total pulmonary infarctions were reduced from 16 per cent in a series of 513

lar fibrillation or flutter ventricular tachycardia or intraventricular block and (8) diabetic acidosis obesity previous pulmonary embolism varicosities of the lower extremities and thrombophlebitis

As a background for the discussion of good and poor risk patients it is worthwhile to review the literature on acute myocardial infarctions prior to the use of anticoagulant therapy TABLE 8 summarizes the mortality from acute myocardial infarction during the first four weeks after the diagnosis in a total

TABLE 9—*Effect of Anticoagulant Therapy in Patients with Acute Myocardial Infarction*

	Good Risks			
Author	*Control Group*		*Treated Group*	
	Patients (No)	*Mortality (%)*	*Patients (No)*	*Mortality (%)*
Wright AHA Study [5]	65	1 5	114	1 8
Russek [207]	611	3 4	—	—
	Poor Risks			
Wright AHA Study [5]	3	97 [illegible]	45	19 4
Russek [207]	[illegible]0	60	—	—

of 3 008 patients The mortality rate varies from 16 to as high as 78 per cent Russek presents data from a study of 1 318 patients in whom anticoagulant therapy was not used Comparison of the series by Russek with the results obtained in the cooperative study of the American Heart Association is shown in TABLE 9 This comparison clearly demonstrates that anticoagulant therapy is most effective in poor risk patients Russek s mortality rate is higher than that of Wright in the poor risk non treated patients because of the older ages of patients in Russek s study Therefore some variation in the poor risk patients in previous reports probably accounts for the difference in mortality rates shown in TABLE 8

Evans [70] has presented data on a series of patients (1 000) who suffered an acute myocardial infarction and who were observed

studies suggested the effectiveness of heparin and more recent studies[118] indicate that infarction does not necessarily lead to scar formation Clinical reports[31] subsequently suggest that anticoagulant therapy has a favorable effect in acute myocardial infarction Accordingly a cooperative study was made by the American Heart Association This study revealed that the mortality and thromboembolic complications were reduced by approximately 50 per cent in patients with acute myocardial

TABLE 8—*Mortality from Acute Myocardial Infarctions (During the First Four Weeks) after Diagnosis*

Authors	*Patients (No)*	*Mortality (%)*
Levine and Brown (19[illegible]8)[1 1]	14[illegible]	53
Connors and Holt (1930)[48]	284	16
Harrington and Wright (1933)[95]	148	54
Master (1936)[135]	26[illegible]	1
Master (1937)[134]	140	[illegible]1
Bland and White (1941)	[illegible]00	19
Levine and Rosenbaum (1941)[1]	[illegible]08	33
Woods and Barnes (1941)[68]	128	4
Newman (1946)[161]	[illegible]0	[illegible]8†
Mintz and Katz (194[illegible])[14]	5[illegible]	22
Yater (1948)[281]	866	52

F t my d l f ct
† Y g d lt
‡ You g ad lts fo r w ek f ll w p

infarctions in whom anticoagulant therapy was used[7] TABLE 7 summarizes the effect of anticoagulant therapy on the mortality rate in acute myocardial infarction[5, 6, 41]

Subsequently it has been pointed out by Russek et al[04] that patients with myocardial infarction run a variable course and on the basis of retrospective studies these patients can be divided into good and poor risks The following criteria for the recognition of poor risk cases have been established (1) previous myocardial infarction (2) intractable pain (3) extreme degree or persistence of shock (4) significant enlargement of the heart (5) gallop rhythm (6) congestive heart failure (7) auricu



FIG. 10—Mural thrombi in the heart of a patient who died from cardiac arrhythmia and who had been on long term Dicumarol therapy for nine years. (Reproduced by permission of Dr. E. Sterling Nichol, Miami, Fla.)

a recognized recurrence of thromboembolism while on anticoagulant therapy.

Acute Arterial Embolization or Occlusion

The use of anticoagulants in arterial occlusions (other than coronary and cerebral) is indicated and the prognosis depends to a large extent on the promptness with which therapy is started.[185, 64, 26] Heparin for immediate antithrombotic effect, fibrinolysin to lyse the clot and oral anticoagulants to prevent re-formation of the clot represent the ideal medical approach

for varying periods of two to 10 years He presents a total mortality rate of only 19 per cent in these patients all of whom were not treated with anticoagulants Reclassification of his data on the basis of good and poor risks reveals that 206 of his patients were in the poor risk category and that the mortality rate in these patients was 40 per cent This again emphasizes the good prognosis for good risk patients even without anticoagulant therapy

One remaining question about good and poor risk classification is Can patients be grouped accurately at the onset of an acute myocardial infarction? One study [9] by a group of experienced cardiologists indicates that 5 6 per cent of the patients required a revision of their classification from good to poor risk The percentage of error by a general physician would probably be somewhat higher therefore it is questionable whether an accurate classification can be made within the first 18 hours of an acute attack A recent report on hospital wards [0] indicated a mortality of 47 per cent in both treated and nontreated patients with acute myocardial infarction This suggests the importance of factors other than anticoagulants in the treatment of this disorder [9, 99, 1, 1, 134, 196, 68]

Another approach to the evaluation of anticoagulant therapy is from postmortem data on patients dying shortly after the onset of clinically diagnosed myocardial infarction These studies reveal that only 50 per cent of patients who die from acute myocardial infarction have thrombi in their coronary arteries and that many have hemorrhage [60] into arteriosclerotic plaques causing the thrombosis [8, 1, 7, 153, 180]

In a recent study [1 0] mural thrombi were found in 44 per cent of the patients treated with anticoagulants and the data indicated that therapy was not effective unless started within three days of onset of infarction It is interesting to note that a patient who had been treated with Dicumarol for nine years following myocardial infarction had a large mural thrombus (See FIGURE 10) However he died from cardiac arrhythmia and never had

TABLE 10—Co tinue l

	Cerebral Thrombosis	*Cerebral Embolus*	*Cerebral Hemorrha₀c*
Incidence	(‹0–80 Per Cent)	(5–› Per Cent	(8–1› Per Cent
Cheyne Stokes or labored respira tion	Seldom	Rare	Common
Conjugate devia tion of eyes	Seldom	Rare	Frequent
Quadriplegia	Rare except in thrombosis of bas ilar artery	Rare	Rare
Stiff neck	Rare	Rare	Frequent
Bilateral extensor plantar response (positive Babinski)	Rare	Rare	Frequent
Leukocytosis	Uncomm n	Uncommon unless embolus is in fected	More than ›0 per cent of patients have over 12 000 leukocytes per cu mm often greater than 20 000
Cerebrospinal fluid	Fluid usually clear pressure slightly increased but not above ° 0 cm of water There may be slight pleocytosis and an increase in protein content	Fluid may be clear or xantho chromic There may be moderate pleocy tosis and increase in protein content especially if em bolus is septic	Usually bloody and under in reased pressure Bloody fluid diag nostic of hemor rhage nto ven tricular or sub arachn›i space Fluid xanthochro mic if hemorrhage is old rarely may le clear if hemor rhage is deep in brain tissue or walled off

TABLE 10—*Differential Diagnosis of Cerebral Thrombosis Cerebral Embolus and Intracerebral Hemorrhage*

	Cerebral Thrombosis	*Cerebral Embolus*	*Cerebral Hemorrhage*
Incidence	(70–80 Per Cent)	(5–22 Per Cent)	(8–15 Per Cent)
Age groups	Same as for hemorrhage	As majority of cases occur from rheumatic hearts most patients are young adults or early middle aged	Same as for arteriosclerosis (increasing with each decade over 50)
History and general physical examination	Same as for hemorhage but blood pressure normal in majority of cases may be elevated	Rheumatic heart disease with mitral stenosis bacterial endocarditis auricular fibrillation recent myocardial infarction emboli elsewhere as in lungs arms legs kidneys or mesentery usually at least one of these pathologic states can be found	Evidence of arteriosclerosis in retinal or peripheral vessels or other evidence of disease of cardiovascular system blood pressure often elevated the systolic pressure usually above 200 mm Hg
Onset	Difficulty in speaking and weakness of arm or leg are the usual first symptoms onset may be gradual or sudden	Very sudden abrupt development of neurologic signs	Severe headache nausea and vomiting frequently occur at onset followed by coma
Coma	Often none at all if present usually less than 24 hours	Coma not usual unless embolus is large	If coma persists more than 24 hours hemorrhage more likely
Convulsions	Occur in per cent of patients at onset	May occur	Occur in 14 per cent of patients at onset

WRIGHT I S Strokes—D g os d mod t tm t I Diagnosis Mod Co pt
I Ca 1 asc la D se se 8 519 1959 R p od t by pe m f th p blish

established but several case reports emphasize that a cautious approach is indicated.[19,16]

The basic question relative to the general effectiveness of anticoagulant therapy in strokes is currently under cooperative study. Preliminary data indicate that fatal hemorrhages occur more frequently in strokes than in other thrombotic conditions when anticoagulants are employed. Further studies will determine if the risk of this therapy is greater than its benefits.

at the present time[150] The question of therapy to prevent recurrent thrombosis or embolism will be discussed further in the chapter on long term anticoagulant therapy Patients with rheumatic heart disease who have auricular fibrillation have frequent embolic episodes and have been shown to benefit from long term anticoagulant therapy There were 55 embolic episodes in a group of 51 patients while on anticoagulant therapy and 197 episodes during a similar period when anticoagulant therapy was withdrawn[4] These findings have been confirmed[9,10]

Cerebral Vascular Disease

The use of anticoagulant therapy in cerebral vascular disease demands an accurate clinical diagnosis since its use in patients with intracranial hemorrhage may be quickly fatal The fear of gross intracerebral bleeding has resulted in a slow and cautious use of anticoagulants[80,90,1,1,74] The differential diagnosis of cerebral thrombosis embolism or hemorrhage can be determined in most instances by utilizing the data presented in TABLE 10 Anticoagulant therapy has been shown to be effective in patients with intermittent insufficiency of the vertebral basilar arterial system or carotid arterial system thrombosis of the vertebral basilar system with infarction and actively advancing occlusion of the carotid artery Attacks of intermittent insufficiency were controlled in 9[illegible] per cent of 179 patients and the rate of mortality from thrombosis was reduced from 50 per cent in 48 control patients and to 7 per cent in 138 patients treated with anticoagulants[14]

McDevitt et al[138] have presented data on the frequency of cerebral emboli in patients with various types of heart disease They found 122 cerebral emboli in 90 patients not given anticoagulants during 2 812 patient months and only 16 cerebral emboli during 2 291 patient months when anticoagulant therapy was used The decision as to whether severe hypertension is a contraindication to anticoagulant therapy has not been definitely

established but several case reports emphasize that a cautious approach is indicated.[1,3,4,6]

The basic question relative to the general effectiveness of anticoagulant therapy in strokes is currently under cooperative study. Preliminary data indicate that fatal hemorrhages occur more frequently in strokes than in other thrombotic conditions when anticoagulants are employed. Further studies will determine if the risk of this therapy is greater than its benefits.

6 Long Term Anticoagulant Therapy

THE MAJOR EFFECT of anticoagulant therapy in patients with acute *myocardial infarction is a reduction in the incidence* of mortality and thromboembolic complications in poor risk patients This effect is probably due to the prevention of recurrent thrombosis or mural thrombi or at least to the prevention of mobilization of localized thrombi These results emphasize the fact that anticoagulant therapy is prophylactic and not curative Therefore it is not surprising that this type of therapy has been tried in patients with recurrent episodes of thromboembolism [17 0 50 51 55 8 79 100 117 130 1 164 167 169 1 1 17 183 00 10 16 17 6 8 33 34 38 41 1 58 67]

RESULTS OF OTHER STUDIES

In 1944 Nichol started long term anticoagulant therapy in a patient who had had three serious myocardial infarctions in 15 months In a series of 5 patients Nichol reported that long term anticoagulants were of definite benefit[168] Peters et al[1 4] also suggested that anticoagulant therapy was of some aid in preventing recurrent coronary thrombosis

Since these preliminary reports many publications have appeared which tend to support the belief that long term anticoagulant therapy is effective and practical The problem of evaluation of this therapy however is more difficult than that involved in the use of anticoagulants in acute thrombotic episodes because the factors implicated in long term experiments are innumerable Again no conclusive therapeutic investigations have been reported but some indication of the effectiveness of long term anticoagulant therapy can be obtained from a review of some of the larger series discussed in this chapter TABLE 11 summarizes the effects of long term anticoagulant therapy in arteriosclerotic heart disease [7 6 116 13 1 173 3 55]

In a small series of patients who had clinical evidence of

congestive heart failure the use of long term anticoagulants was found to reduce the mortality rate from 13 to 7.5 per cent and some decrease in the incidence of thromboembolic complications was noted

Owren[173] followed 106 patients who had survived an attack of acute myocardial infarction for at least eight weeks and

TABLE 11.—*Results of Long Term Anticoagulant Therapy in Arteriosclerotic Heart Disease*

Author	*Disease*	*Control Group*		*Anticoagulant Group*	
		Cases (No.)	*Mortality (%)*	*Cases (No.)*	*Mortality (%)*
Anderson (1950)[7]	CHF	150	13.3	147	7.5
Owren (1953)[174]	MI	(No controls)		106	4/yr
Waaler (1954)	Angina	(No controls)		266	12.4
Suzman (1955)[23]	MI	89	33	82	7.3
Keyes (1956)[114]	MI	234	48	121	9.9
Bjerkelund (195)	MI	118	35.6	119	20.1
Manchester (1957)[131]	MI	200	34	204	14.2
Nichol (1959)[165]	Angina	10	40	96	6.2
	MI	407	37.3	995	19.5
Pickering (1959)[186]	MI	188†	17	195	11

CHF congestive heart failure MI postmyocardial infarction † Low dosage of anticoagulant therapy served as the control group

treated these patients with anticoagulants for approximately two and six tenths years He noted a yearly mortality rate of 4 per cent or a total mortality rate of 8.5 per cent He had no controls but indicated that this was about one half the mortality rate one would expect from a review of the literature

Waaler reported a careful study of 266 patients with angina pectoris who were treated with anticoagulants for two and one half years The mortality rate in these patients was 12.4 per cent however he had no controls The study indicated that patients with a short history of angina were benefited most by the treatment

In a controlled investigation Suzman et al [23] reported on the effect of anticoagulant therapy carried on for three to 76 months

in a series of patients who had been given anticoagulant therapy during the acute phase of their myocardial infarction In 82 patients treated with long term anticoagulants there was a mortality of 7.3 per cent in 89 controls the mortality was 33 per cent In comparing both groups of patients with severe acute attacks the mortality was 9 per cent in the group treated and 46.7 per cent in the control group

Keyes et al [115, 11] have presented data on a series of 121 patients who were treated and 234 control patients after myocardial infarction The mortality was 9.9 per cent and 18 per cent respectively A few patients observed for four years in whom there was a history of a single attack had a mortality of 8.4 per cent in the group treated and 41 per cent in the control group In patients with multiple myocardial infarctions who were observed for four years there was a mortality of 12 per cent in the group treated and 62 per cent in the control group

Manchester [131] has followed a controlled series of patients after acute myocardial infarction for a period of one to 10 years and found a mortality rate of 14 per cent in those treated compared to 34 per cent in the control group

Nichol et al [16] have presented the combined results of 10 different cardiologists who used long term anticoagulant therapy in private patients The mortality rate in 995 patients treated was 12.5 per cent compared to a control group of 407 patients with a 37 per cent mortality Anticoagulant therapy was used for less than one year in 12 per cent of the patients treated and the mortality rate was 28 per cent in 319 patients who stopped anticoagulant therapy

Bjerkelund [6] has presented his findings in a carefully controlled series of 237 patients who were followed for 66 months There was a mortality of 20 per cent in those treated and 35.6 per cent in the control group The statistical analysis of the data revealed a rather interesting finding namely that anticoagulant therapy was of greater benefit to patients who were good risks than to those who were poor risks This has recently been confirmed in the cooperative study [186] from Eng

land but contrasts with the findings in acute myocardial infarction when anticoagulants were used

RISK OF HEMORRHAGE

The major criticism against anticoagulant therapy is the possibility of hemorrhage Its occurrence is reported in every series in which the patients were kept on long term therapy The risk of hemorrhage increases with the length of treatment and the level of depression of coagulation factors An accurate estimate of the risk is not possible from published reports Bjerkelund has summarized the data from the literature and found 22 deaths

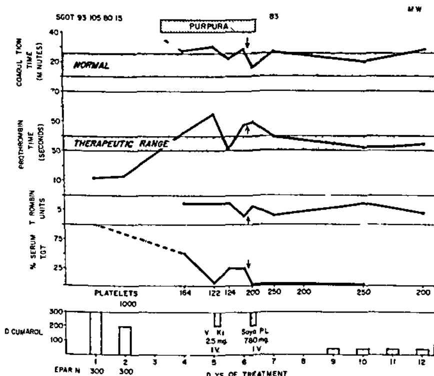


FIG 11—Effects of vitamin K and intravenous soy bean phospholipid on the various coagulation tests on a patient with purpura from Dicumarol therapy From Boyles et al Blood 14 81 1959 reproduced by permission of the publisher

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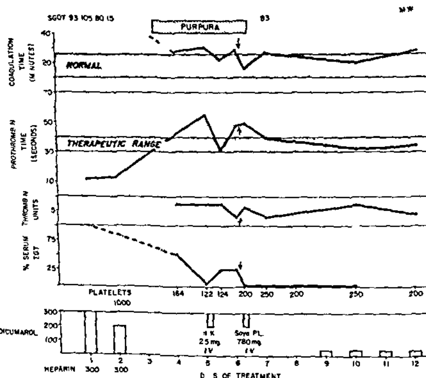


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Keyes et al [116, 117] have presented data on a series of 121 patients who were treated and 234 control patients after myocardial infarction The mortality was 9 9 per cent and 48 per cent respectively A few patients observed for four years in whom there was a history of a single attack had a mortality of 8 4 per cent in the group treated and 41 per cent in the control group In patients with multiple myocardial infarctions who were observed for four years there was a mortality of 12 per cent in the group treated and 62 per cent in the control group

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7 *Thrombolytic Therapy*

BLOOD CLOTS are temporary structures in the normal process of hemostasis The clot serves as a framework for new tissue formation and the fibrin is gradually replaced by dissolution Intravascular thrombi are morphologically different in arteries and veins Those in arteries are generally crescent in shape and relatively acellular with very little tendency toward retrograde progression which is thought to be related to the higher pressure in the artery Venous clots are usually filled with entrapped cellular elements especially platelets and there is a marked tendency toward retrograde progression Arterial occlusion is a much more serious disorder than venous thrombosis therefore the need for an agent to restore circulation quickly is more important in this condition The enzyme responsible for normal lysis of the clot is called fibrinolysin Methods of activation of fibrinolysin its clinical implication and therapeutic use are discussed in this chapter

The physiologic activation of fibrinolysin is almost as complex as the clotting reactions and the exact mechanisms involved are unknown However the nature of the reactions involved can be summarized briefly into three general reactions (1) Fibrinolysin is an enzyme which will act specifically on fibrinogen or fibrin so that the fibrinogen will not clot upon the addition of thrombin and the fibrin will become soluble (2) Fibrinolysin is formed from its inactive precursor profibrinolysin by the enzymatic action of an activator (3) This activator can be formed by many diverse methods In general activation of the activator can be classified into either in vitro or in vivo methods

One of the earliest observations in blood coagulation was the demonstration of proteolytic activity in serum after the addition of chloroform ether or thymol [1] Later Tillett and Garner [44] found that human plasma clots dissolved rapidly in the presence of certain strains of beta hemolytic streptococci In 1941 Mil

(006 per cent) from long term anticoagulant therapy in 3 773 patients treated The over all estimated mortality from all types of anticoagulants is 0 2 per cent while the incidence of major bleeding is between 5 to 20 per cent in most of the larger series reported

Daily capsules of vitamins C and P are beneficial for the many patients on long term anticoagulants in whom bruising is observed[39] Effects of phospholipid administered intravenously on Dicumarol induced purpura is shown in FIGURE 11

BLOOD COAGULATION AND CORONARY ARTERIOSCLEROSIS

Many authors have reported considerable differences in the prognosis of patients with coronary heart disease[1 1 7 9 43 47–49 57 63 81 104 1 136 143 1 7 170 1 8 193 9 49 56 57 61 66] The cause of coronary artery *disease is generally considered to be* arteriosclerosis the etiology of which is unknown An interesting theory postulates that coronary arteriosclerosis is secondary to repeated intramural coronary thrombi[3 60 6 69 97 98 139 14 147 179 11 6 80 81]

Histologic studies have shown a relationship between intimal hemorrhage and fatty changes in the arterial wall[149] Several recent observations tend to support this old theory Diets low in fat in Norway and Russia during World War 2 were associated with the reduced incidence of thromboembolic diseases[153 1 4] Fatty meals have been reported to accelerate clotting[169 170 43 2 9] and also inhibit fibrinolysis[89] Decreased fibrinolysin[10 1] accelerated[140] clotting and delayed heparin clotting times[18] have *been observed in patients with acute myocardial infarctions* The common factor related to these findings is not known but if these studies have any merit anticoagulant therapy may in some indirect manner inhibit the progressive development of coronary arteriosclerosis These observations may also explain the beneficial effect of intermittent heparin on patients with angina pectoris[67 68] In any event these findings are of interest and deserve further investigation

either streptokinase or staphylokinase depending on the bacterial source of the kinase

Other studies demonstrated inexplicable species differences [1, 4, 15] when fibrinolysin was activated with streptokinase namely human preparations of profibrinolysin were rapidly activated with streptokinase whereas bovine profibrinolysin was not These findings have been explained by the suggestive evidence that a proactivator exists in human plasma which combines stoichiometrically with streptokinase The activator then enzymatically catalyzes profibrinolysin from any species into fibrinolysin The fibrinolysin in turn acts as a proteolytic enzyme on fibrinogen and fibrin [3] The reaction products have similar electrophoretic patterns (See FIGURE 12) The various body fluids i e urine saliva tears and milk also contain an activator which functions like the activator found in human blood [3]

In addition to the activation of profibrinolysin by the method just described many studies have shown that tissue extracts[3, 13, 83] can activate a proteolytic system in the blood This tissue extract acts directly Physical characteristics of the activators from tissue extracts and from blood or body fluids indicate that the two agents are of different chemical structures However technical difficulties in obtaining tissue extract free from trace amounts of blood or serum render a definite statement impossible (See DIAGRAM 5 for the activation of fibrinolysin)

Under certain conditions the circulating blood becomes capable of rapidly liquifying fibrin It is not clear if the mechanism is the same in each condition but the result appears to be an in vivo activation of profibrinolysin to fibrinolysin These conditions are accidental death drowning trauma ischemia fear electroshock prolonged bleeding pyrogen reactions and certain neoplastic diseases especially carcinoma of the prostate [15] In addition there are certain clinical situations in which hemorrhage occurs with low levels of fibrinogen in the circulating blood such as takes place with infusion of amniotic fluid into the circulation and extensive pulmonary surgical procedures One explanation offered is that the accumulation of thrombo

stone [146] showed that purified fibrin resisted the lytic action of streptococcal culture filtrates but the addition of small amounts of human globulin resulted in rapid lysis Subsequent studies suggested that the factor in human globulin fraction was a proteolytic enzyme precursor which was activated by the addition of bacterial filtrate The agent in the filtrate was called

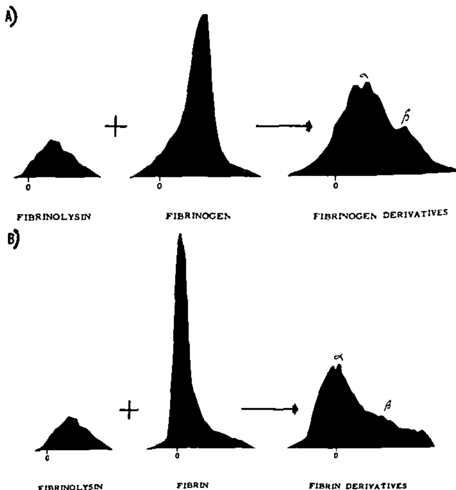


Fig 1?—Electrophoretic patterns of fibrinolysin fibrinogen and fibrin and the reaction products of the enzyme fibrinolysin on either fibrinogen or fibrin

attack most proteins Streptokinase and bacterial polysaccharides activate profibrinolysin and are effective via the activation of fibrinolysin an enzyme which specially attacks fibrinogen and fibrin Clinical studies of these various agents are discussed individually

Trypsin

Trypsin is one of the pancreatic enzymes which catalyzes the hydrolysis of proteins in the intestinal tract The pancreas forms the inert precursor trypsinogen which is activated by the intestinal enzyme enterokinase to trypsin with subsequent autocatalytic activation of the remaining trypsinogen by trypsin The enzyme can be prepared in crystalline form and studies with synthetic substrates indicate that its action is specifically for peptide bonds with a free carboxyl group and either arginine or lysine at the site of hydrolysis In addition trypsin is able to split certain specific amides and amino acids which have been used to assay the enzyme activity

In vitro studies have shown that trypsin in low concentrations accelerates blood clotting and in higher concentrations inhibits clotting and catalyzes the activation of profibrinolysin to fibrinolysin In vivo studies by Mirsky and Freis[148] demonstrated that trypsin administered intravenously caused liver and kidney damage with extensive intramuscular clotting Theories on the mechanism of trypsin in accelerating clotting indicate that the enzyme accelerated the formation of thrombin from prothrombin either directly or by the activation of plasma thromboplastin In any event this is an important contraindication to the use of intravenous trypsin in the treatment of intravascular thrombi

Other studies have shown that high doses of intravenous trypsin produce a shock syndrome which in many respects is similar to the intravenous administration of thrombin These toxicity reactions can be reduced by slowing the rate of the *infusion of trypsin however no free enzyme activity could be* demonstrated presumably due to the rapid inactivation of trypsin by antitrypsin inhibitors in the plasma Despite these find

plastic tissue [107, 109] materials in the blood leads to activation of the circulating profibrinolysin and slow progressive intravascular clotting hence the circulating fibrinogen is depleted

Inhibitors in the blood tend to inactivate circulating fibrinolysin thus a relative increase in fibrinolytic activity can occur with a decreased level of antifibrinolysin [4] This substance and other inhibitors to the activation of profibrinolysin limit the level to which an increase in fibrinolytic activity may rise in

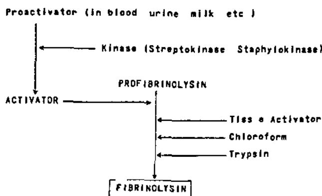


DIAGRAM 5—Activation of fibrinolysin

the blood but they also complicate any attempt at quantitative estimation or study of fibrinolytic reactions Therefore evaluation of the effectiveness of the enzyme is difficult In addition the inhibitors complicate the problem of purification of fibrinolysin and the activator The inactivation of fibrinolysin is considered to occur by the formation of a complex with antifibrinolysin *which can be partially split with an agent such as* chloroform

THROMBOLYSIS BY VARIOUS ENZYMES

Five agents have been shown to have an in vivo dissolving action on fibrin [131, 14, 144, 1?, 37] These are trypsin chymotrypsin streptokinase pyrogen free bacterial polysaccharide and fibrinolysin Trypsin and chymotrypsin are proteolytic enzymes which

due to the reaction of the organism to the breakdown products of rapidly destroyed proteins

Clinical studies with chymotrypsin have indicated that its activity is similar to trypsin The toxicity produced also appears similar with generalized urticaria and pain at the injection sites tending to limit its usefulness

Pyrogen free Polysaccharide

The mode of action of bacterial lipopolysaccharides is pre sumed due to its ability to activate profibrinolysin to fibrinolysin by in vivo febrile reaction [14] Although this type of therapy does not appear useful in most instances of intravascular clotting it does give some indication of one method by which fibrinolysin may be activated physiologically Interestingly the increased fibrinolytic activity is not blocked by prednisolone or by control of fever with aspirin [144]

Streptokinase

Streptokinase is interesting because of its ability to produce fibrinolytic activity both in vitro and in vivo [112] Other bacteria such as staphylococci can also produce this action but because the mechanism of action is apparently similar and because strep tokinase has been most extensively investigated this discussion will center on this agent Studies by Tillett et al [246] have shown that beta strains of streptococcus produce the most effective fibrinolytic agent Numerous experiments have been made to purify the active agent and small quantities of rather pure prepa rations have been studied

The mechanism of action is not definitely established but reports indicate that streptokinase is not fibrinolytic in itself A proactivator is considered to be chemically combined with streptokinase forming an activator which enzymatically converts profibrinolysin into fibrinolysin Studies have shown some species difference Purified preparations of human profibrino lysin are rapidly activated with streptokinase while bovine prepa

ings Innerfield[108] *reported in 1952 that intravenously administered* trypsin would lyse experimentally produced clots in animals These findings were not confirmed by subsequent investigations from several different laboratories In addition intravenous trypsin in animals caused changes in the electrocardiogram Thus these contraindications and the nonspecific nature of the proteolytic action of trypsin has resulted in its abandonment generally for the treatment for intravascular clotting

Chymotrypsin

Chymotrypsin like trypsin is a pancreatic enzyme which is activated by trypsin into several forms which differ in their relative proteolytic and hydrolytic activity The active enzymes are all formed from inert chymotrypsinogen which is produced in this state by the pancreas The outstanding differences in activity from those of trypsin are its ability to clot milk but not to clot blood Chymotrypsin requires the presence of an aromatic ring containing tyrosine or phenylalanine residue for its hydrolytic activity but it will attack a wide range of synthetic *substrates and proteins the latter being acted upon at different* linkages from those attacked by trypsin

Tagnon[40] has shown that intravenous chymotrypsin will not cause intravascular clotting but will produce a shock syndrome similar to that produced by trypsin His studies demonstrated a fall in fibrinogen and a prolongation of the prothrombin time which he considered to be a direct attack on these coagulation proteins Jensen et al[111] found that chymotrypsin *lowers the* antifibrinolytic titer of the blood which may explain the action on fibrinogen Later studies have confirmed the in vivo action on fibrinogen and some thrombolytic activity but a rapid rise in inhibitor activity suggested that this enzyme had little potential use in intravascular thrombi Another difference in chymotrypsin from trypsin is that the shock phenomenon cannot be inhibited by heparin which may indicate that this *toxicity is*

siderably in different patients even if the amount of profibrinolysin is similar this is related to variation in the levels of antifibrinolysin FIGURE 13 illustrates the electrophoretic pattern of the interaction of purified fibrinolysin and antifibrinolysin In addition streptococcal infections result in an inhibitor to streptokinase which is a rather frequent occurrence In some patients the level of this inhibitor is sufficient to require extremely high doses of streptokinase in order to obtain an effective level of this agent for the activation of profibrinolysin Since even the purest preparations of streptokinase produce toxicity in a significant number of patients the amount that can be infused is limited Chills fever hypotension and allergic phenomena are the most frequently observed toxic reactions However another theoretic danger is hemorrhage for once the fibrinolytic system has been activated in vivo there is no known specific method which will reverse the activity If a very active fibrinolytic system is present it will lyse all of the circulating fibrinogen which could be treated with human Fraction I but this preparation is contaminated with proenzyme which would further increase the lytic activity At the present time the best *therapy is plasma which contains fibrinogen and profibrinolysin* but plasma also contains the inhibitor antifibrinolysin

Despite these drawbacks streptokinase has been used successfully in many patients with various thrombotic conditions If preparations of streptokinase can be made in which the activity is retained and the toxic reactions are eliminated it would undoubtedly be used more widely in the treatment of all types of intravascular clotting

Fibrinolysin

Fibrinolysin is the naturally occurring enzyme formed from plasma profibrinolysin Recent advances in the purification of human profibrinolysin have resulted in the successful application of in vitro activated enzyme for the lysis of arterial and venous thrombi in animals and man[14 4 15] FIGURE 14 demon

rations are not affected The addition of small quantities of human globulin or profibrinolysin however results in the rapid activation of the mixture of streptokinase and bovine profibrinolysin This is thought to be due to a proactivator in human plasma which is missing from the blood of other species It has also been suggested that human profibrinolysin combines with streptokinase more readily and this is the activator of profibrinolysin in any species

Other in vitro studies have shown that fibrinolysin attacks fibrinogen and fibrin at an equal rate while in vivo studies have



FIG 13—Electrophoretic pattern of fibrinolysin antifibrinolysin and the inactive complex formed from their interaction

shown that fibrin appears to be destroyed more selectively than fibrinogen These studies however are complicated by inhibitors in the plasma which limit the value of any in vitro estimate of circulating fibrinolytic activity and the rapid turnover rate of fibrinogen in the intact animal This difference in the rate of attack is probably explained by some adsorption of fibrinolysin onto the fibrin clot and by the rapid replacement of the destroyed circulating fibrinogen

In the clincal use of streptokinase the level of circulating profibrinolysin and the antifibrinolysin are limiting factors in the quantity of active fibrinolysin produced in the circulation The activation of profibrinolysin occurs within one half hour and within one hour nearly all of the circulating profibrinolysin is converted to fibrinolysin This is revealed by the failure of additional quantities of streptokinase to produce further fibrinolytic activity The level of fibrinolytic activity may vary con

that segmental injection of fibrinolysin is necessary to achieve effective in vivo lysis of coronary thrombi

FIGURE 21 illustrates the effect of intravenous fibrinolysin on the coagulation factors in a woman with cerebral thrombosis However all fibrinolysin preparations have been associated with systemic reactions such as fever chills and hypotension in a significant percentage of the patients In addition skin reactions have been noted in some instances with repeated admin

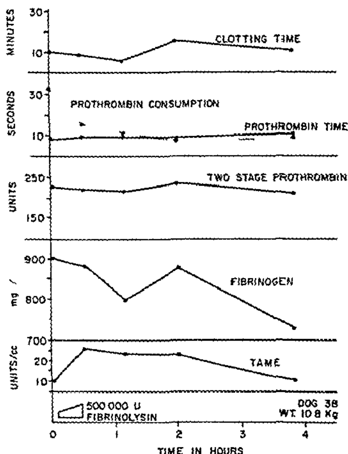


Fig. 15—Effect of intravenous fibrinolysin therapy on the coagulation factors in a dog after an induced blood clot

strates the in vitro inactivation of the esterase activity (fibrinolytic) with various plasmas

Animal studies and preliminary studies in man indicate that fibrinolysin is a specific and effective agent for the lysis of intravenous clots FIGURES 15 and 16 show the effect of intravenous

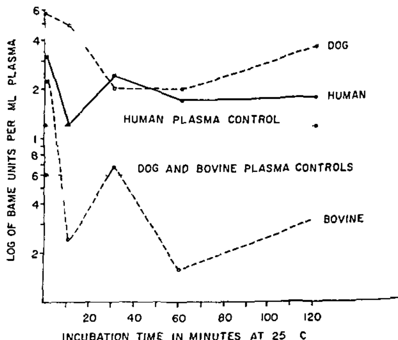


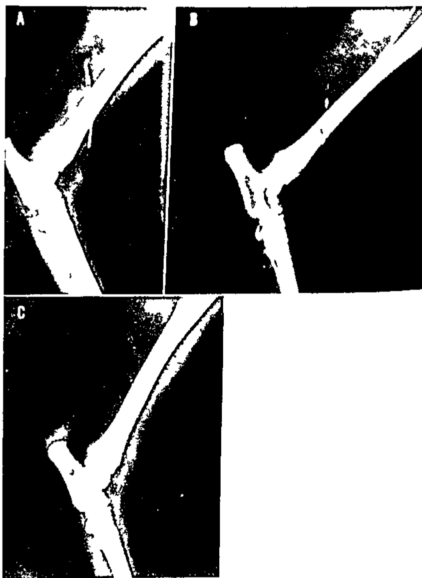
FIG 14—In vitro inactivation of fibrinolysin by normal plasma as determined by measurements of the synthetic substrate BAME

fibrinolysin on the coagulation factors and the accelerated lysis of radio-opaque venous clot [33] in dogs The gross appearance of an induced radio opaque coronary thrombus in a dog is illustrated in FIGURE 17 Serial x ray examinations in FIGURE 18 illustrate the failure of large amounts of intravenous human fibrinolysin to accelerate the lysis of this induced clot The amount of fibrinolysin used in this experiment was much larger than could be used clinically as illustrated by the marked effects on the blood factors shown in FIGURE 19 These findings suggest



FIG. 1.—Induced radio-opaque blood clot in a branch of the left descending *coronary artery at surgery.*

istration of the enzyme. Fibrinolysin has been shown to be effective in the lysis of clots even without evidence of a marked rise in measurable fibrinolytic activity. This may be due to the absorption of the enzyme onto the fibrin clots. Another explanation may be the difficulty of measuring fibrinolytic activity because of the inhibitor antifibrinolysin. It is important to note that the enzyme is ineffective once the clot has been covered with endothelium, which occurs within three to seven days. This limits the use of fibrinolysin to only recent thrombotic episodes. The enzyme can be administered in conjunction with oral anticoagulants, and it seems likely that fibrinolysin combined with anticoagulants will be prescribed for all types of acute throm



Fic 16—Ly is of a iduced ra li j ique bl i l cl t in a dog ve n after sy tem fibrinolysin admini tratı n From B yle J I I Cl Me l (in pres) reproduced b permi sion of the pul li her

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tension which with large doses that must be used with intravenous therapy Preliminary observations indicate that fibrinolysin administered under pressure into the root of the aorta or into the carotid artery is the most effective method for the lysis thrombi in the coronary or cerebral arteries



Fig 18B—After closure of the chest wall and just before the administration of intravenous fibrinolysin therapy

botic episodes when a *nontoxic preparation becomes available*. FIGURE 22 demonstrates the serial changes in the x-ray examinations of a patient with pulmonary embolism who was treated with intravenous fibrinolysin and anticoagulant therapy. At the present time, however, its use is limited by the frequency of systemic reactions such as fever, chills and occasional hypo-



FIG. 18A–C—Serial x-ray appearance of a radio-opaque induced coronary artery clot in a dog.

FIG. 18A (above)—At the time of surgery.

tension which with large doses that must be used with intrave nous therapy Preliminary observations indicate that fibrinoly sin administered under pressure into the root of the aorta or into the carotid artery is the most effective method for the lysis thrombi in the coronary or cerebral arteries



Fig 18B—After closure of the chest wall and just before the administration of intravenous fibrinolysin therapy



Fig. 18C—Twenty hours following intravenous fibrinolysin therapy

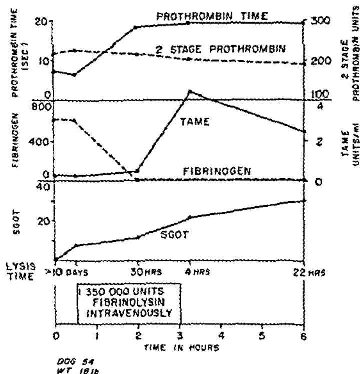


Fig 19—Effects of a large intravenous infusion of fibrinolysin on the coagulation factors in a dog with an induced coronary thrombosis Increased in the prothrombin time fibrinolytic activity (TAME) lysis time and transaminase activity are noted along with a decrease of circulating level of fibrinogen to zero From [illegible] reproduced by permission of the publisher



FIG 20—Partial lysis of a radio opaque coronary blood clot in a dog with dissemination of the clot to the terminal branches of the artery

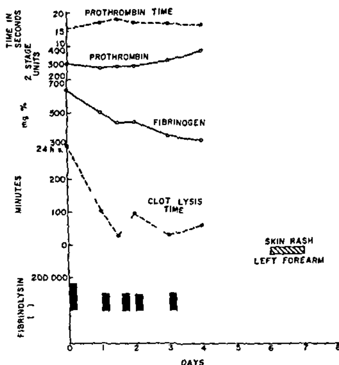


FIG. 21—Effects of repeated intravenous infusions of human fibrinolysin on the various coagulation factors in a patient with cerebral thrombosis. From Boyles, J. Florida M. A. (in press), reproduced by permission of the publisher.



Fig 22A–F—Series of x rays of a patient with a pulmonary embolus who was successfully treated with intravenous fibrinolysin therapy

Fig 22A (above)—X ray taken five days before the onset of acute chest pain



Fig. 2 B—X ray at the time of acute chest pain

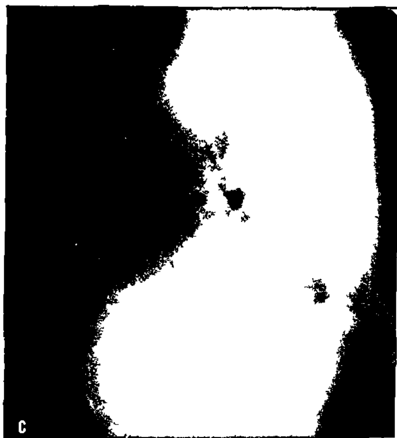


Fig. 29C—Right lateral at the time of acute chest pain



Fig 99D—Portable x ray taken 0 hours after the first infusion of intravenous fibrinolysin



Fig. 21.—Portable x ray taken 24 hours after the second infusion of intravenous fibrinolysin



Fig 22F—X ray taken nine days after the onset of acute chest pain the patient appeared completely recovered clinically

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Addendum

The most important factors in the study of blood coagulation are the attainment of (1) A carefully obtained blood sample one which is uncontaminated with tissue thromboplastin with out air bubbles and with prompt efficient admixture of anti coagulant (2) clean glassware (3) daily standardization of reagents and (4) attention to seemingly trivial details

The methods described in this section are not original but

SYNONYMS FOR BLOOD COAGULATION FACTORS

	†	*
Factor I	Fibrinogen	
Factor II	Prothrombin	Prothrombase Thrombozyme
Factor III	Thrombin	Thrombase
Factor IV	Tissue thromboplastin	Thrombokinease Cytozyme Thrombokinase
Factor V	Ac Globulin (plasma accelerator globulin)	Labile factor Proaccelerin Thrombokinase Accelerator factor Component A of prothrombin Co factor of thromboplastin Prothrombinokinase Proprothrombinase Prothrombogenase Plasma prothrombin conversion facto
Factor VI	Serum Ac Globulin	Accelerin Serum accelerator Thrombokinase Thrombogenase Prothrombinas

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SYNONYMS FOR BLOOD COAGULATION FACTORS—*Continued*

*	†	‡
Factor X	PTA (Plasma thromboplastic antecedent)	Plasma thromboplastic factor C—PTF—C
—	Platelet thromboplastic factor	Thromboplastinogenase Platelet activator Thromboplastic cellular component TCC

* Suggested name by International Committee on Nomenclature of Blood Clotting Factors
† Nomenclature used in this book
‡ Infrequently used names

Calcium ions are needed for all reactions of blood coagulation

Other Factors Thought to be Concerned with Plasma Thromboplastin Formation

Factor X
Plasma thromboplastic factor D PTF—D
Spaet's IV factor of thromboplastin
Platelet factors 1 2 3 4
HF (Hageman factor)
Stuart factor (Prower factor)
Car factor

Inhibitors of Coagulation

Heparin
Antithrombin—thrombin inhibitor—(Antithrombin I II III and IV)
Antithrombin co factor
Profibrinolysin—plasminogen—tryptogen—prolysin
Fibrinolysin—plasmin—trypiase—lysin—lytic factor
Proantifibrinolysin
Antifibrinolysin
Circulating anticoagulants—antithromboplastins (antibodies) thromboplastin inhibitor—antithrombokinase—heparin like substances—heparin co factor

represent slight modification of previously described methods which have proved to be reliable These methods have been compiled in this simplified form for convenience

Paper electrophoresis has proved to be a convenient method for determining the effectiveness of various coagulation protein purification procedures This is illustrated in FIGURE 23

SYNONYMS FOR BLOOD COAGULATION FACTORS—*Continued*

	†	‡
——	pro SPCA	pro convertin
Factor VII	SPCA (Serum prothrombin conversion accelerator)	Stable factor Autoprothrombin I Convertin Co factor V Component B of prothrombin Serum accelerator Prothrombin accelerator Prothrombin conversion factor Prothrombin converting factor Serozyme Co thromboplastin Prothrombinogen Kappa factor (chickens)
Factor VIII	AHG (Antihemophilic globulin)	Antihemophilic globulin A AHF—Antihemophilic factor Prothrimbokinase Plasmakinin Thromboplastinogen Thrombocytolysin Thrombokatilysin Thromboplastic plasma component—TPC Plasma thromboplastic factor Plasma thromboplastic factor—PTF—A Alpha prothromboplastin Platelet co factor I PTF—plasma thromboplastic factor Facteur antihemophilique A
Factor IX	PTC (Plasma thromboplastic component)	Facteur antihemophilique B Plasma factor X Christmas factor Beta prothromboplastin Platelet co factor II Autoprothrombin II Thromboplastin B Antihemophilic globulin B plasma thromboplastic factor B—PTF—B

by the administration of large doses of ACTH and less effectively with Cortisone

BLEEDING TIME

Purpose To establish an index of platelet function and tissue factors involved in hemostasis

Method Cleanse the ear lobe with alcohol and allow to dry Make a small puncture through the skin with a lancet Bard Parker blade or needle Do not touch the skin or apply any external pressure At 30 second intervals blot all the blood which has flowed with a piece of filter paper

Interpretation Normal bleeding time is one to three minutes When the platelets are reduced or the fibrinogen content of the blood is low bleeding may continue for an hour or more When bleeding is prolonged the twentieth blot may be fully as large as the first

Comments Prolonged bleeding time is observed in primary usually congenital defects of the vascular wall (pseudohemophilia) and when there is a qualitative or quantitative platelet deficiency The mechanism by which thrombocytopenia produces prolonged bleeding time is not completely understood This is usually attributed to a lack of vasoconstrictor substance (serotonin) from the platelets agglutinating at the site of vascular injury and to a lack of mechanical plugging by the platelets

The prolonged bleeding time of thrombocytopenia may be corrected partially by the continued administration of ACTH and temporarily corrected by the administration of platelets

CLOTTING TIME

Purpose Clotting time is used as a general index of the sum of the various coagulation reactions which take place when blood clots

Method Place 1 cc of whole blood into each of three tubes and note the clotting time Tilt tube No 1 every 60 seconds until a solid clot forms Then tilt tube No 2 and after the blood

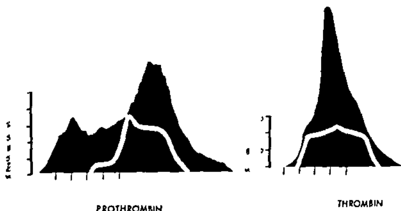


FIG 23—Correlation between the electrophoretic pattern and the specific activity of purified prothrombin and thrombin as determined with electrophoretic technics

TOURNIQUET TEST

Purpose To measure capillary fragility

Method Outline an area 5 cm in diameter on the anterior aspect of the arm Place a sphygotometer cuff around the patient s arm and inflate the cuff to a pressure half way between systolic and diastolic or at least 100 mm pressure Maintain this pressure for five minutes after which time release the pressure and remove the cuff Wait a few minutes and then inspect the arm for petechiae

Interpretation In normals 0 to 10 petechiae may be found If more are present abnormal capillary fragility is indicated

Comments This test is positive in patients with increased vascular fragility which occurs in scurvy vascular purpura of undetermined origin senile purpura etc In all of these conditions the positivity of the test indicates only the inability of the vessel wall to resist stress

The test is also positive in thrombocytopenic purpura The mechanism is unknown but the defect can be corrected by the administration of platelets It can also be corrected promptly

viable platelets Physical factors also like the weight of the clot nature of the surface etc will influence clot retraction

WHOLE BLOOD LYSIS TIME

Purpose To test for possible increase in fibrinolytic activity in a blood sample

Method The clots in test tubes used for testing clotting time and clot retraction may be observed for 24 hours in a water bath at 37 C Lysis of clots faster than 12 hours is significant of increased fibrinolytic activity or poor clot formation caused by a defective coagulation process or a reduced fibrinogen content Abnormal results can be evaluated by more quantitative methods described later

PLATELET COUNT

Purpose To quantitate circulating platelets

Materials 1 Platelet fluid

Sequesterene—Na (recrystallized 3 x)		1 0 Gm
Sodium chloride		1 7 Gm
Distilled water	to	100 cc

The solution is filtered through filter paper and stored in the refrigerator The solution should be filtered each time before using

2 Bard Parker blade No 11

3 U S certified hemocytometers and pipets

Method With this method blood can be collected from the finger tip or from a vein It is based on the use of an anticoagulating solution which has the ability of preserving the viability of platelets to the maximum The method employed is essentially similar to that used for the determination of the red cell count

Count from Finger Tip Blood Cleanse the finger tip of the patient with alcohol and wipe dry Puncture the finger tip and discard the first drop of blood Draw blood into the hemocytometer pipet to the 0 05 mark and aspirate platelet fluid to the 1 01 mark (as for a red blood cell count) Mix the contents

has clotted tilt tube No 3 The clotting time of tube No 3 is taken as the significant time Marked variation in the clotting time of the three tubes usually indicates poor technic

Interpretation Normal clotting time ranges between 10 and 15 minutes Prolonged clotting time is found when the following conditions exist hemophilic diseases (AHG PTC PTA) severe heparinemia fibrinogenopenia or severe prothrombinemia decrease in accelerators of prothrombin conversion decreased platelet concentration pseudohemophilia and circulating anti coagulants

CLOT RETRACTION OF WHOLE BLOOD

Purpose To determine the function of platelets in regard to clot retraction

Method Collect blood with the two syringe technic dis carding the blood in the first syringe Remove the needle from the second syringe and transfer 2 cc of blood to a glass test tube which should be kept in a water bath at 37 C Examine the test tube at the *end of one hour after completion of clotting*

Interpretation The clot from normal blood will retract and serum will collect between the clot and wall of the test tube The serum can be poured off and measured The volume of serum obtained multiplied by 50 represents the clot retraction in per cent Direct visual observation however is usually suffi cient to distinguish between normal and abnormal clot retrac tion

Normal clot retraction of whole blood is 20 to 60 per cent This retraction is generally a direct function of the number of active platelets present No clot retraction is observed in blood and plasma of patients with severe thrombocytopenia or throm boasthenia Clot retraction can be restored to normal tempo rarily in these patients by the administration of viable platelets

Comments The degree of clot retraction is influenced by a number of factors (1) the fibrinogen content of the blood (2) the volume of plasma and most important (3) the number of

Method Place 0 2 cc oxalated plasma and 0 2 cc of 025 M CaCl in a test tube Let this mixture stand in the water bath at 37 C for 60 seconds and observe every 15 seconds thereafter The end point is the time fibrin strands appear and a clot forms

Interpretation The normal for the test is 90 to 120 seconds with platelet rich plasma and 120 to 240 seconds with platelet poor plasma If the test is prolonged it is supplemented by a titration of unknown plasma against normal plasma to determine whether the normal plasma s clotting time is prolonged by the addition of unknown plasma The prolongation of the clotting time of normal plasma by the unknown plasma would indicate a circulating anticoagulant

ONE STAGE PROTHROMBIN TIME

Purpose To estimate prothrombin in plasma

Method Collect 4 5 cc whole blood and add to 0 5 cc of 0 1 M potassium oxalate Spin at 3 000 x G for 10 minutes to obtain platelet poor plasma Aspirate plasma from the cells then add the following in Lee White tubes in the order given 0 1 cc plasma 0 1 cc thromboplastin and 0 1 cc CaCl (025 M) Test clotting time from the addition of CaCl Place the tube in the water bath at 37 C for 10 seconds then tilt tube until a clot forms Do this procedure in duplicate

Interpretation Normal prothrombin time means the prothrombin Ac globulin SPCA and fibrinogen are of normal content in the plasma If there is a reduction in any or all of these factors the clotting time is prolonged The 100 per cent prothrombin time is 11 0 to 13 0 seconds When the one stage prothrombin time reaches a level of 25 per cent or less hemorrhage may occur

A prothrombin percentage curve can be made up with prothrombin free plasma or saline Values are different especially in the 0 to 20 per cent range therefore the shape of the two dilution curves is different This is done as follows

the pipet well by repeated inversion an charge and ordinary red blood cell counting chamber as for a red blood cell count After charging set the chamber aside for 15 to 20 minutes so that platelets can settle To prevent evaporation cover the chamber with a Petri dish containing a piece of moistened filter paper Count the platelets under high dry magnification with a 10 x eyepiece Count all platelets in 25 squares (each square is divided into 16 individual squares) To obtain the absolute number of platelets per cu mm multiply the number of platelets counted by 2 000 (the figure of 2 000 is the result of a multiplication of the dilution 200 x the depth of the chamber 1 mm)

Count from Venous Blood Aspirate sterile platelet fluid into a tuberculin syringe to the 0 1 cc mark through a 23 gauge needle Then draw venous blood to the 1 0 cc mark Transfer the mixture to a siliconized test tube Collect blood from the test tube into the pipet The rest of the procedure is identical *to that described for the finger tip method*

In calculating the absolute number of platelets an additional correction factor should be introduced namely 5/3 (1 66) which indicates the dilution of venous blood with platelet fluid during its collection

Comment For more precise counts the use of phase microscopy is advised A 43 x phase objective with a long working distance condenser and 10 x eyepiece can be used This method is very useful in recognizing the platelets clearly It is necessary to count the platelets within one hour after charging the chamber in order to prevent formation of platelet clumps This formation greatly reduces the accuracy of the method

The normal platelet count is 200 000 to 300 000 per cu mm *with the method described*

RECALCIFICATION TIME OF OXALATED PLASMA

Purpose This is an exclusion test for circulating anticoagulants provided defects in platelets and plasma thromboplastin formation are excluded

BEDSIDE PROTHROMBIN TIME (MANCHESTER)

Purpose To determine prothrombin converting factors in whole blood

Materials Thromboplastin (0 15 Gm dried rabbit brain) microscopy slide with a well and a stop watch

Method Add thromboplastin to 4 cc of saline incubate the solution at 40 C for 20 minutes and centrifuge Place 20 cu mm of thromboplastin solution on a clean microscope slide and then add 20 cu mm of whole blood from finger tip prick Mix and start stop watch

Interpretation Clotting time is indicated by a thick viscous clot Normal clotting time is 15 to 18 seconds Room temperature is a important variable in this test and a control determination must be done each time this test is performed Dilution curves can be done but are only gross estimates of the relative percentages of prothrombin converting factors

PROTHROMBIN CONSUMPTION

Purpose To measure the prothrombin remaining in serum one hour after clotting

Method Place 1 cc whole blood in a test tube and after clotting put the tube in a water bath (37 C) for one hour In a second tube add the following in the order given 0 1 cc fibrinogen solution 0 1 cc thromboplastin 0 1 cc CaCl and 0 1 cc serum from the clotted blood (free of red cells as they contain thromboplastin) Time is checked with the addition of the serum Place in the water bath for 10 seconds tilt until a clot is formed The clot is the end point

Interpretation Prothrombin should be utilized when blood clots If there is a defect in the clotting mechanism the clotting time of this test is short indicating an abnormal amount of prothrombin remaining in the serum A shortened clotting time occurs when there are decreases in platelet number or function hemophilic diseases or circulating anticoagulants

This serum prothrombin time is compared to the plasma

ONE STAGE PROTHROMBIN DILUTION CURVE

Normal Plasma (cc)	Deprothrombonized Plasma or Saline (cc)	Prothrombin (%)
0 5	—	100
0 45	+0 05	90
0 4	+0 10	80
0 35	+0 15	0
0 30	+0 20	60
0 25	+0 25	50
0 20	+0 30	40
0 15	+0 35	30
0 10	+0 10	20
0 05	+0 45	10
0 0[illegible]	+0 [illegible]	5

U 0 1 cc f abo e d lut n + 0 1 c th mbopla t + 0 1 cc C d perf m a Q k ne t g p th m t

OWREN WARE STRAGNELL PROTHROMBIN TEST

Purpose To determine prothrombin

Materials Anticoagulant—0 1 M of potassium oxalate with 100 micrograms of heparin per cc

Method Add 4 5 cc of blood to 0 5 cc of the anticoagulant Centrifuge the mixture for 10 minutes and then remove the plasma Dilute the plasma 1 10 (1 cc to 9 cc of distilled water) All reagents should be warmed to 37 C Add 0 1 cc of prothrombin free beef plasma * or 0 5 per cent bovine fibrinogen (fraction I) † to 0 1 cc of diluted test plasma in a small test tube (10 mm x 75 mm) Then add 0 1 cc of a thromboplastin calcium mixture (potent thromboplastin mixed with an equal volume of 025 M calcium chloride)

Interpretation The 100 per cent standard clotting time is 25 seconds the 20 per cent standard clotting time is 45 seconds Read from log paper the plot of clotting time and per cent of standard

* Hyland Laboratory product
† Armour product

intervals until clotting occurs in the reaction mixture and 30 seconds thereafter pipet 0 1 cc of plasma saline-calcium chloride mixture into the fibrinogen solution and record the clotting time

Interpretation In hemophilia there is a prolonged latent period but the total amount is unaffected A thrombocytopenic curve goes up and down in various peaks Blood from a patient on oral anticoagulants has a prolonged latent period and the total amount of thrombin generated is reduced The normal range is 8 to 12 units of prothrombin generated within 300 seconds

THROMBIN UNITS

C T	Units	C T	Units	C T	Units	C T	Units
15	23 5	54	[illegible]	89	4 6	182	2 4
18	19 5	55	1	91	4 5	18	2 3
22	10 6	56	05	92	4 5	190	2 3
23	10 55		0	93-4	4 4	200	2 2
24	10 5	58	6 8	96	4 3	203	2 2
25	10 5	9	6 5	98	4 2	211	2 1
[illegible]	10 45	60	6	100-3	4 1	224	2 0
28	10 3	61	6 6	104-	4 0	225	2 0
30	10 2	62	6 4	10	3 9	233	1 9
31	10 2	63	6 3	10	3 8	236	1 9
32	10 15	64	6 2	111	3 5	244	1 8
34	10 1	6	6 1	112	3	245	1 5
36	10 0	66	6 0	11	3 6	260	1
37	10 0	6	9	120	3 5	265	1 6
38	9 5	68	5 [illegible]	125	3 4	280	1 7
41	9 4	69	5 8	12	3 3	[illegible]	1 55
43	9 0	71	5	130	3 2	310	1
44	8 8	3	5 6	138	3 1	320	1 45
45	8 6		4	142	3 0	322	1 4
47	8 2	78	2	145	2 8	360	1 3
48	8 1	9	5 1	158	2 5	412	1 2
49	8 0	80	5 0	160	2	436	1 1
50	8	81	5 0	163	2 65	460	1 05
51	7 7	82-3	4 9	166	2 6	489	1 0
52	7 6	84-7	4 8	170	2 5	500	1 0
3	7 5	85	4 7	1 8	2 45	501	0 0

Clotting time is given in seconds

prothrombin time and the difference is expressed in seconds Normally the difference is always greater than 20 seconds i e at least 80 per cent of the prothrombin is consumed as measured with the prothrombin curve used in calculating the one stage prothrombin activity The normal value is usually 27 seconds and above when there is a relatively normal amount of prothrombin present in the plasma

Comment In patients with hemophilia or hemophilia like disease and idiopathic thrombocytopenic purpura (ITP) the prothrombin activity of serum is high (i e a rapid prothrombin time) Abnormal prothrombin consumption in ITP is not corrected by the administration of ACTH or Cortisone nor by the infusion of bank blood It is corrected temporarily by the administration of concentrated platelets In this condition as in hemophilia the test is useful in evaluating the clinical status of the patient and the effectiveness of therapy This test is of little value in patients who are on oral anticoagulants because the prothrombin in the plasma is greatly reduced and the amount remaining in the serum is small and below the sensitivity of this method

GENERATION OF THROMBIN IN RECALCIFIED PLASMA

Purpose To measure the time required for the peak of generation and the total amount of thrombin generated

Solution One hundred mg of fibrinogen * per 10 cc of borate buffer

Method Centrifuge 4 5 cc of blood and 0 5 cc of citrate at 1 000 r p m for two minutes or allow mixture to settle in order to obtain platelet rich plasma Place 0 4 cc of fibrinogen solution in each of 20 tubes which should be kept in a water bath at 37 C In another tube place the following 1 0 cc of patient s platelet rich plasma *1 0 cc of 0 85 per cent NaCl and 1 0 cc of* CaCl Start to time the reaction (If patient s recalcification time is prolonged use twice the strength of CaCl) At 60 second

Bovine Fibrinogen Fraction I Armour Laboratories

tors Temperature All dilutions and undiluted reagents should be kept refrigerated until ready for use

Method The test is performed at 37 C Into each of 6 test tubes is placed 0 1 cc of normal substrate plasma (oxalated) A thromboplastin generating mixture is made by adding 0 3 cc of

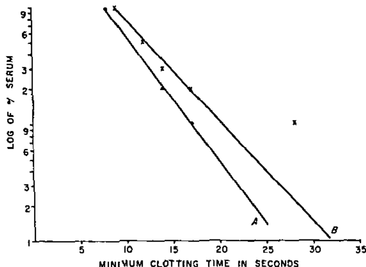


FIG 24—Effects of progressive dilution of normal serum in the thromboplastin generation test which can be used to determine the relative percentage of thromboplastin generated

each of the following (1) prothrombin free plasma (2) serum (3) platelet suspension or phospholipid substitute and (4) 0 025 M CaCl At one minute intervals after the addition of the calcium solution 0 1 cc aliquots of the thromboplastin generating mixture is added simultaneously with 0 1 cc of 0 025 M CaCl into one of the tubes containing 0 1 cc of the plasma substrate at 37 C Clotting times are determined by the usual one stage prothrombin technique A normal control must be performed with each preparation of reagents

THROMBOPLASTIN GENERATION TEST

Purpose To measure intrinsic plasma thromboplastin

Materials (1a) Preparation of platelets Utilizing siliconized equipment 20 cc of citrated blood is quickly chilled to 5 C and centrifuged at 1 000 x G for five minutes and aspirate the platelet rich plasma This plasma is centrifuged at 3 000 x G for 30 minutes then one half of the supernatant is removed and replaced with saline This procedure is repeated three times with fifteen minute periods of centrifugation The platelets are suspended in saline to a final volume of 2 cc (or at least one fourth of the original plasma volume)

(1b) Platelet substitutes for thromboplastin generation test Brain phospholipid Three hundred mg of brain phospholipid gum is homogenized in 50 cc of 0 85 per cent NaCl This is the stock solution For thromboplastin generation the stock solution is diluted 1 50 with saline

Soy bean phospholipid * The stock solution is 50 mg per cc For the thromboplastin generation test 0 025 mg per cc is used Both brain or soy bean phospholipids may be substituted for platelets in the thromboplastin generation test To replace platelet suspension 0 3 cc of either brain or soy bean phospholipid is used

(2) Prothrombin free plasma One cc of a 0 15 M $BaSO_4$ suspension is centrifuged at 3 000 r p m for 15 minutes the supernatant fluid is discarded One cc of oxalated plasma is mixed with the $BaSO_4$ sediment and is incubated at 37 C for 10 minutes The supernatant from the $BaSO_4$ plasma mixture is diluted 1 5 with saline just before being used in this test

(3) Serum Serum aged at 37 C for six hours is diluted 1 10 with saline

(4) Substrate Plasma Plasma used as substrate should be within the normal range for prothrombin content In addition it should contain at least adequate amounts of accelerator fac

Glidden and Company Chicago Illinois

oxalated plasma (15 to 25 days old) loses its content of Ac globulin This is added to supply the fibrinogen and prothrombin If a small amount of Ac-globulin is added to the unknown plasma the test reads longer than the one stage prothrombin determination and indicates a deficiency of AcG

SPCA

Purpose To see if there is a deficiency of SPCA when the result of the one stage prothrombin test is abnormal

Method Mix the following in the order given 0 01 cc of aged stored serum 0 09 cc of patient s plasma 0 1 cc of thromboplastin and 0 1 cc of CaCl Test the clotting time and continue as a regular one stage procedure

Interpretation If the serum reduces the prothrombin time to within normal limits (60 to 100 per cent) there is probably a deficiency of SPCA

SPCA (PROCONVERTIN) ASSAY

Purpose To determine SPCA (proconvertin)

Materials 1 Proconvertin free beef plasma Collect nine parts of whole bovine blood in one part potassium oxalate monohydrate 2 5 per cent Separate plasma by centrifugation Place five ordinary filter pads 12 5 cm in diameter in the bottom of a glass Buchner funnel of the same diameter Above them put 15 Gm of powdered wood charcoal and above this put another filter paper or a layer of gauze to prevent admixture of charcoal with plasma This filtering layer is pretreated by allowing 20 cc of 0 85 per cent saline to pass through under suction in order to pack the layer of the charcoal Then place 50 cc of bovine plasma in the funnel and allow to filter by gravity only Two or three passages of the same plasma through the same carbon are sufficient to produce essentially proconvertin free plasma After the last filtration suction is used for removal of a relatively large amount of plasma soaked in the charcoal All procedures are done at 4 C The prothrombin content is determined by the

Interpretation If the clotting time of the patient s factors falls in the normal range (9 to 12 seconds) after six minutes incubation it can be assumed that the patient s AHG and serum factors are normal If *the clotting time is in the doubtful* or abnormal range (16 to 18 seconds) the test procedure should be carried out substituting one at a time the patient s factors in place of the reagent factors

When the patient s AHG is substituted for reagent AHG and *the clotting time is above 18 seconds* after six *minutes incubation* an AHG deficiency can be assumed

When the patient s serum is substituted for reagent serum and the clotting time is above 18 seconds after six minutes incubation a serum factor deficiency can be assumed (i e PTC PTA Stuart Prower Hageman or Car factor) The specific serum defect can be identified by mutual correction studies with known congenital deficient serum and patient s serum If the patient s plasma and serum are both doubtfully abnormal this suggests a circulating anticoagulant or antithromboplastin The effects of progressive serum dilutions on the thrombolplastin generation is illustrated in FIGURE 24

ACCELERATOR GLOBULIN (STEFANINI S METHOD)

Purpose To measure the amount of Ac globulin in oxalated plasma

Method Draw 4 5 cc of whole blood and add it to 0 5 cc of 025 M oxalate Spin down at full speed for 10 minutes (platelet poor plasma) and aspirate plasma using Lee White tubes Mix the following in the order given 0 01 cc of unknown plasma 0 09 cc of stored plasma (15 to 25 days old at 5 C) 0 1 cc of thromboplastin and 0 1 cc of CaCl Test clotting time from the addition of the CaCl Place in the water bath at 37 C for 10 seconds Tilt tube until a clot forms

Interpretation A short clotting time means the plasma contains an adequate amount of Ac globulin When the amount reaches 25 per cent or less hemorrhage may occur Stored

centrifuged the supernatant is discarded Ten cc of the fibrinogen saline solution is added to each of the 20 tubes The mixture is incubated with stirring at 37 C for 10 minutes and then centrifuged Fifty cc of cold (5 C) 100 per cent saturated $(NH_4)_2SO_4$ are added to the supernatant The tubes are centrifuged at 5 C and the supernatant is discarded The precipitate is dissolved in saline solution This $(NH_4)_2SO_4$ purification process is repeated three times The final solution is dialyzed for six hours—changing the saline solution after the first hour

2 Reaction Mixture This mixture is prepared by adding the following

0 9 cc regular thromboplastin
0 1 cc beef serum diluted 1 10 with saline
2 0 cc acacia buffer mixture

Any active thromboplastin works well The acacia buffer mixture is prepared by adding 20 cc of 15 per cent crude acacia 5 cc of imidazole buffer and 35 cc of 0 85 per cent NaCl This mixture is stable indefinitely in the deep freeze

3 Thrombin One ampul of Topical Thrombin (1 000 units) * is dissolved in 5 cc of distilled water and 5 cc of glycerol is added to stabilize the solution This mixture should be stored in the refrigerator

4 Defibrinated plasma Plasma is defibrinated by placing the following in a test tube

0 5 cc fresh oxalated plasma
0 4 cc 0 85 per cent NaCl
0 1 cc thrombin (100 units)

The clot is collected on a rod as soon as it appears The defibrinated plasma is allowed to stand for at least 10 minutes to let the antithrombin inactivate the added thrombin

5 Defibrinated plasma dilution Dilutions are estimated by the results from the one stage prothrombin time *i e* plasma with a prothrombin time of 12 seconds is usually diluted 1 50

* Parke Davis and Company product

two stage assay and should be greater than 75 per cent of the original concentration

2 Testing plasma Collect human blood with ordinary glass syringes and needles and add 4 5 cc to a tube containing 0 5 cc of 0 1 M sodium oxalate Separate plasma by centrifuging at 4 000 r p m for five minutes in a small angle centrifuge

3 Diluting solution Make diluting solution with 800 cc of 0 85 NaCl solution add 200 cc of veronal buffer and 700 mg potassium oxalate monohydrate

4 Buffer Make veronal buffer by adding to 570 cc of 0 1 M sodium diethylbarbitrate 430 cc of 0 1 M HCl and 5 67 Gm NaCl The pH is measured and adjusted to 7 35 to 7 40

Method Add the following in the order listed and note the clotting time 0 2 cc proconvertin free beef plasma 0 2 cc of 1 10 dilution of the test plasma (0 1 cc plasma + 0 9 cc diluting solution) 0 2 cc of rabbit brain thromboplastin and 0 2 cc of CaCl (0 03M) Do control with normal plasma in the same manner described above and compare with prepared dilution curve

The percentage of SPCA can be assayed with this method from a dilution curve prepared in a similar manner to that described for the one stage prothrombin test using dilutions of normal serum

Interpretation The sources of errors are in the dilution of the test plasma and detoration of the prothrombin in the proconvertin free beef plasma The percentage of SPCA is determined on the normal control done with the same reagents

TWO STAGE PROTHROMBIN TIME

Purpose To determine prothrombin quantitatively

Materials 1 Purified Fibrinogen Solution Six Gm of commercial fibrinogen (Bovine) * is dissolved in 200 cc of saline solution at 5 C Twenty centrifuge tubes (15 cc tubes) each containing 2 cc of $BaSO_4$ solution (500 mg $BaSO_4$/cc) are

* Bovine fibrinogen Fraction I Armour Laboratories Chicago Illinois

BIURET METHOD OF FIBRINOGEN

Purpose To quantitate fibrinogen

Solution Mehl s biuret solution is prepared by mixing the following 100 cc of ethylene glycol 40 cc of 60 per cent NaOH and 50 cc of 4 per cent $CuSO_4$ 5H O Dilute mixture to 100

FIBRINOGEN CURVE
540 WAVE LENGTH—BIURET METHOD

Optical Density	*Fibrinogen (mg)*
0 015	50
0 030	100
0 0 0	1 0
0 065	00
0 080	2 0
0 095	300
0 110	350
0 1 5	400
0 14	4 0
0 160	500
0 175	550
0 190	600
0 0	6 0
0 2 0	700
0 35	750
0 250	800
0 270	8 0
0 285	900
0 300	9 0
0 310	1000

cc with distilled water and heat until precipitation is complete then filter Add sufficient NaOH to make a 10 to 11 per cent solution

Method Place 1 cc of citrated plasma (0 5 cc citrate per 4 5 cc blood) in a 100 cc beaker and add 20 cc of distilled water To this solution add 1 0 cc of 025 M CaCl and 1 0 cc of thrombin (100 units per cc) Remove the fibrin clot by winding on a glass rod Wash the clot three times with distilled water and then place it in 4 0 cc of Mehl s biuret solution Allow clot to digest in the biuret solution for 12 hours Then dilute the solu

with saline and a plasma with a prothrombin time of 25 seconds is diluted 1 10

Method One cc of diluted plasma and 3 cc of reaction mixture are combined Zero point four cc of this combination is added to 0 1 cc of fibrinogen solution This test is usually done at 29 C Clotting time is tested every 30 seconds until optimum time has been reached which must be between 12 to 18 seconds or the test must be repeated at a different dilution Daily control is performed in order to determine the activity of the reagents The percentage normal is determined from the daily control

Calculation

THROMBIN UNITS

C T	*Units*	*C T*	*Units*
12 0	1 34	15 0	1 00
12 5	1 26	15 5	0 96
13 0	1 20	16 0	0 93
13 5	1 14	16 5	0 89
14 0	1 09	17 0	0 86
14 5	1 04	17 5	0 83
		18 0	0 80

C T is clotting time seconds

Total Thrombin units =

Thrombin units of assay X plasma dilution X 5+

X anticoagulant dilution

+ 1 5 dilution because 0 4 cc of the reaction mixture is added to 0 1 fibrinogen solution

Interpretation Since prothrombin is rapidly activated to thrombin in the presence of optimal amounts of accelerators the two stage test measures primarily the quantity of thrombin (prothrombin) in a given solution This is in marked contrast to the one stage prothrombin time determination which primarily measures the velocity of the prothrombin activation Normal plasma contains 300 to 100 units of prothrombin

One unit of fibrinolysin is the amount necessary to digest a 0 3 per cent fibrin clot in two minutes at 37 C in an imidazole buffered system at pH 7 2 This test not only measures fibrinolysin but also includes a partial estimation of the rate of activity If a dilution is used correct for it by means of the following calculation

Units of fibrinolysin per cc = 5 x CF x D (D = dilution CF = correction factor for deviation from 120 seconds in the lysis time)

Comment The sources of error are fibrinogen and thrombin contamination Bovine fibrinogen is the most suitable reagent Less difficulty will be encountered if purified fibrinogen similar to that used in the two stage prothrombin assay is used Human fibrinogen preparations are contaminated with fibrinolysin to a much greater degree If human fibrinogen must be used a 0 15 per cent clot instead of the 0 3 per cent bovine clot will give a unit approximately equal to the defined unit Fibrinolysin or profibrinolysin contaminates thrombin* but since bovine profibrinolysin activates poorly with streptokinase the only source of error to be considered is the active proteolytic enzyme

ESTIMATION OF FIBRINOLYTIC ACTIVITY (FIBRIN PLATE METHOD OF ASTRUP AND MULLERTZ)

Purpose To determine fibrinolytic activity giving an accurate assay with a wide variety of materials

Materials (1) Petri dishes—10 cm diameter with uniform and level surfaces (2) Fibrinogen—diluted with 0 85 per cent NaCl to a concentration of 0 2 or 0 4 per cent Use purified fibrinogen † preparation of 80 to 90 per cent clottable protein

* C[illegible] thro[illegible] (Parke Davis and Co) can be ad orbed with barium sulfat[illegible] that to adsorb plasma will result in a relatively pure (electr[illegible]) preparation

† [illegible] thrombin [illegible] a [illegible] venient [illegible] urce [illegible] fibrinogen

tion to 10 cc with distilled water Read on the Coleman photometer (540 mμ) and compare to a standard curve

Standard Fibrinogen Curve Weigh out bovine fibrinogen (20 mg per cc) and make dilutions of the fibrinogen Determine the amount of protein by the method described above

To determine the amount of clottable protein perform the following steps Add 1 cc of fibrinogen solution to 0 1 cc of thrombin (10 units per cc) Weigh glass rod and wind clot out on rod Place rod with clot in an oven at 85 C and dry over night or desiccate Then weigh the clot

Interpretation (a) *Weight of the clot* — by 20 = *per cent* of clottable protein

(b) Per cent of clottable protein x weight of protein in the various dilutions = mg of fibrinogen Use optical density obtained with various dilutions and plot curve as illustrated below Normal range is 300–600 mg per cent

FIBRINOLYSIN ACTIVITY OF SERUM

Purpose To determine the amount of fibrinolysin in human serum

Materials (1) Human serum (2) bovine fibrinogen (purified) 0 6 per cent clottable in imidazole buffered saline solution (3) bovine thrombin 75 U per cc in 50 per cent glycerol (4) open ended capillary tubes (5) test tubes 10 x 75 mm (6) pipets (7) water bath at 37 C (8) stopwatch

Method If plasma is to be tested convert the plasma to serum by recalcification or the addition of thrombin Aspirate the serum from the fibrin clot and test

Place 0 2 cc of thrombin and 0 2 cc of serum or suitable dilution into a 10 x 75 mm test tube Quickly add 0 5 cc of 0 6 per cent bovine fibrinogen and immediately start a stopwatch Tilt or shake the test tube to hasten the mixing of the reagents When a clot forms insert a capillary tube to the bottom of the clot and place in a water bath at 37 C The instant the liquid level in the capillary tube is parallel to the clot level in the test tube the end point has been reached

sin * may be used as a standard of reference As small an amount as 0 02 μg can be detected The method is especially adapted for plasmin (fibrinolysin) but may be used as a practical tool in the study of other fibrinolytic enzymes

ANTITHROMBIN TITER

Purpose To assay antithrombin

Method Incubate 3 cc of serum or plasma for 10 minutes at 56 C Dilute the serum (normally 1 10 to 1 60 with saline) and add 1 cc of diluted serum to 1 cc of thrombin † (4 units per cc) Incubate this mixture at 28 C for *exactly* four minutes and then add 0 5 cc of the mixture to 0 5 cc fibrinogen Note clotting time Normal serum control is determined in the same manner

Interpretation One antithrombin unit (ATU) is defined as that amount which will neutralize one unit of thrombin in four minutes at 28 C The normal range is 74 to 115 ATU and the average is 90 ATU

PROTAMINE TITRATION

Purpose To estimate the amount of heparin activity in a blood sample

Materials Protamine ‡ (50 mg) dissolved in 50 cc of distilled water This contains 1 000 gamma per cc The solution is stable for six days Heparin Sodium 1 000 U S P § units per cc or 10 000 gamma per cc (Resulting heparinized blood contains an added 2 000 gamma per 0 5 cc)

Method Draw 5 cc of blood into a syringe containing 0 1 cc heparin Add this to a tube with 0 1 cc heparin and mix well Place 0 5 cc of this heparinized blood into each of 10 test tubes

Purified human fibrinolysin (Actase—Ortho Pharmaceutical Corporation and Thrombolysin—Merck Sharp and Dohme)

† Parke Davis and Co

Eli Lilly product

§ Product of Upjohn Laboratories

(3) Buffer—add 338 cc of 0 1 M HCl to 662 cc of 0 1 M Sodium diethyl barbiturate and then dilute with 320 cc water (resulting solution pH 7 8 ionic strength = 0 05) (4) Thrombin—purified thrombin * in a final concentration of 100 units per cc in 0 85 per cent NaCl solution (5) Incubator—temperature of 37 C (6) Pipets—5 cc and 0 1 cc graduated in 0 001 cc

Method Using the buffer as diluent (fibrinogen concentration 0 2 or 0 1 per cent) place 9 0 cc of the diluted fibrinogen solution in each Petri dish Then put the Petri dishes on a horizontal surface and clot by adding 0 2 cc thrombin A clot forms it should be even and homogeneous throughout Add *exactly* 0 03 cc of the solution to be tested onto the clotted surface in the form of a drop Usually three single determinations are carried out on a plate Then incubate the plates 18 to 20 hours at 37 C and calculate the product of two perpendicular diameters (square mm) of the digested area Convert the areas into concentrations by interpolation on a reference curve

Calculations Purified enzymes produce a straight line when the logarithm of the product of the area is plotted against the logarithm of the concentrations as expressed in units The following equation applies to this straight line relationship

$$A = k \times c^a \quad \text{or} \quad \log A = a \log c + b$$

where A = area or product of two prependicular diameters c is the relative enzyme concentration a is a constant denoting the slope of the curve and k and b are constants It is possible to compare two solutions only when the curves have identical slopes solutions differing in concentrations produce parallel dilution curves in a logarithmic = log graph By means of such curves the concentration of the unknown solutions can be estimated by interpolation on a dilution curve of a solution used as a standard of reference

Comments In estimating the reproducibility and the sensitivity of the method crystalline trypsin or purified fibrinoly

* Thrombin (Parke Davis and Company) adsorbed with barium sulfate provides a purified preparation of thrombin

Test	*Final platelet dilutions*	*Mixtures*
#4	1: 000	1 cc of 1:1 000 dilution cc CaCl 2 cc NaCl
#5	1:10 000	1 cc of 1:1 000 dilution 1.5 cc CaCl 4.5 cc NaCl
#6	1:50 000	1 cc of 1:10 000 dilution 2 cc CaCl 2 cc NaCl
#	1:100 000	1 cc of 1:10 000 dilution 4.5 cc CaCl 4.5 cc NaCl

Interpretation If there is an antithromboplastin present, it will be neutralized as revealed by prolongation of the clotting time in the patient's plasma as compared with normal plasma.

CIRCULATING ANTICOAGULANT

Purpose To show the presence of a circulating anticoagulant.

Method In order to minimize the surface effects, siliconized vessels and equipment are used for collecting blood samples. Blood is collected from the patient in sequesterene. Platelet poor plasma is made by centrifugation (20 000 G for 10 minutes) and stored in the refrigerator. Platelet poor plasma or 0.85 per cent NaCl is added to 15 test tubes as follows: (1) 0.1 cc saline, (2) 0.5 cc saline, (3) 0.1 cc plasma, (4) 0.2 cc plasma, (5) 0.5 cc plasma, (6) 0.5 cc plasma, (7) 0.2 cc plasma, (8) 0.1 cc plasma, (9) 0.5 cc saline, (10) 0.1 cc saline, (11) 0.1 cc saline, (12) 0.5 cc saline, (13) 0.1 cc plasma, (14) 0.2 cc plasma, (15) 0.5 cc plasma.

Blood is obtained from a normal, a stopwatch is started from the instant of successful venipuncture. One cc of the normal blood is added to each of the 15 test tubes. After careful mixing the tubes are observed for clotting at 37 C.

Interpretation Significant prolongation of the clotting times with platelet poor plasma indicates abnormal anticoagulant activity. Any result with platelet poor plasma greater than the saline controls by at least seven minutes is in the abnormal range.

containing the following amounts of 1 per cent protamine solution

Tube	*Protamine (cc)*
1	0 08
	0 10
3	0 12
4	0 14
5	0 16
6	0 18
7	0 20
8	0 [illegible]
9	0 24
10	0 26

After setting up the tubes shake well and allow to stand for one hour Read the first clotted test tube as the end point

Interpretation The normal range is between the 0 10 and 0 14 tubes Control normal blood should be run with the same reagents daily Between 100 to 140 gamma of protamine will neutralize 200 gamma of heparin

ANTITHROMBOPLASTIN TEST

Purpose To see if there is a circulating antithrombolplastin

Method Make dilutions of thromboplastin with saline and CaCl Add 0 2 cc of the diluted thromboplastin calcium mixture to 0 1 cc of unknown plasma and run a one stage determination Control normal plasma should be performed with the same dilutions

Test	*Thromboplast dilut*	*Mixture*
#1	1 100	[illegible] cc CaCl 1 0 cc NaCl 0 1 cc thromboplastin
#2	1 500	1 cc of 1 100 dilution [illegible] cc CaCl [illegible] cc NaCl
#3	1 1 000	1 cc of 1 100 dil[illegible] 1 cc CaCl 1 cc NaCl

3 Add about 250 cc of chloroform

4 Let this solution sit for 30 minutes stirring occasionally with applicator stick

5 Then filter through a Buchner funnel with suction using double filter paper (Whatman #1)

6 Evaporate on a mild hot plate until a gummy residue forms

7 Reweigh beaker to determine the amount of active material

8 Homogenize in saline (300 mg /50 cc 0 85 per cent NaCl)

PREPARATION OF BRAIN PHOSPHOLIPID

1 Fresh brain stripped of meninges and blood vessels and minus the corpus callosum

2 Hunks of brain are put into acetone in the Waring Blender and blended for 4 to 5 minutes

3 Let the solution settle and decant off the acetone

4 Add more acetone and repeat this procedure about six to 10 times

5 Then filter through a Buchner funnel and wash about six times until cholesterol free

6 Put in a desiccator to dry

7 Store powder in the deep freeze

PREPARATION OF THROMBOPLASTIN SOLUTION

Materials 1 Acetone brain extract

2 Saline (0 85 per cent) solution

3 Phenol (25 mg /10 cc thromboplastin solution)

4 0 1 M potassium oxalate

Method 1 Weigh out 200 to 350 mg dried acetone brain extract

2 Add 10 cc 0 85 per cent saline

3 Incubate at 50 to 54 C for 15 minutes inverting every two minutes once

4 Centrifuge at full speed for five minutes using the supernatant as solution

Possible errors in this test would tend to make an abnormally shortened clotting time due to possible contaminating tissue thromboplastin or surface effect

SOLUTIONS

1 0 025 M CaCl 1 387 Gm in 500 cc distilled water

2 0 1 M Potassium Oxalate 18 4 Gm in 1 000 cc distilled water

3 3 8 per cent Sodium Citrate 7 6 Gm in 200 cc distilled water

4 Sequesterene (used as platelet fluid) 1 75 Gm in 100 cc saline Add a few crystals of brilliant cresyl blue

5 Phosphate Buffer

A M/15 KH PO_4 9 078 Gm in 1 000 cc

B M/15 Na HPO_4 (Anhydrous) 9 47 Gm in 1 000 cc

for pH 6 1 M/15 5 parts of A
1 part of B

for pH 7 8 M/15 1 part of A
10 parts of B

6 Fibrinogen Buffer (Borate) Each liter contains

11 25 Gm H_3BO_3

2 25 Gm NaCl

4 0 Gm Na B_4O_7 10 H O

This solution has a pH of 7 75

7 Protamine 1 per cent (Eli Lilly Co) 50 mg /50 cc distilled water (Stable for six days)

8 Fibrinogen solution (fibrinogen—Bovine from Armour Lab Chicago Ill) 20 mg /2 cc Borate buffer

9 Heparin sodium 1 000 USP units/cc (Upjohn)

10 Sequesterene (Alrose Chemical Company) for platelet preparation 1 75 Gm /100 cc distilled water

PHOSPHOLIPID SOLUTION

1 Weigh beaker

2 Weigh 5 Gm of powdered brain phospholipid

Addendum Bibliography

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5 Add phenol to prevent growth of bacteria

6 Add 0 2 potassium oxalate for each 10 cc to neutralize any calcium

Dried material is potent indefinitely in the frozen state Solution is stable for six weeks or more

THROMBOPLASTIN

Materials 1 Normal brain stripped of vessels and meninges

2 Buchner funnel and flasks

3 Waring Blender and jars

4 At least 30 lbs of acetone

5 Two large glass cylinders

6 Several pieces of large filter paper

Procedure 1 Knead by hand small pieces of brain in three times the volume of acetone Decant acetone repeat the procedure two more times Tissue will feel dry to the hand

2 After decanting the acetone place brain tissue in Waring Blender with three times the volume of acetone (Cup should be one half full)

3 Turn on the blender rapidly for about 1 minute Allow the tissue to settle decant acetone and repeat three more times

4 After decanting wash material in Buchner funnel with acetone and let dry Repeat washings with acetone until material is completely dry (About 6 washings are needed)

5 Spread the dried material on a large sheet of filter paper and make into a powder When odor of acetone is completely gone package with parafin and screw top vial (Store in the deep freeze this is good for at least a year)

Index

(*Note* In addition to regular index entries particularly tests see also technical deta ls indexed under Ad lendum ')

Accelerator globulin 4
  determination one stage 13
  and Dicumarol 5
Accelerin 4
Addendum 95–1 5
  blood coagulation factors synonyms for 95–97
  electrophoresis paper 97
  solutions 1 9–124
    brain phosphol pi l 1 3
    phospholipid 129
    thrombopla tin 124
    thrombor lastin solution 123
  synonyms for blood coagulation fac tors 95–97
  tests 95–12
    accelerator globulin (Stefaninis method) 110
    antithrombin titer 119
    antithromboplastin 120
    bedside prothrombin time (Man che ter) 105
    b uret method of fibrinogen 115
    bleeding time 99
    circulating ant coagulant 121
    clot retraction of whole blood 100
    clotting time 99
    fibrinolysin act v ty of serum 116
    fibr nolyt c activity (fibrin plate method of Astrup and Mullertz) 117
    generation of thromb in in recalci fied plasma 106
    one stage proth omb n t me 103
    Owren Ware Stragnell prothrom bin te t 104
    platelet cou nt 101
    protamine titratic n 119
    proth nbin consumption 10
    recalcification time of oxalated pla ma 102
    SI C A 111
    SPCA (proconvertin) as ay 111
    thromboplastin generation 108
    tourniquet 98
    two stage prc thrombin time 112
    v hole blood lysis time 101
Angina pectoris long term anticoagu lant therapy in 51
Anticoagulant therapy
  in cerebral vascular d sease 48
  complic itions of 1
  discontinuance of 37
  effect of on myocar lial infarct on 41 43
  evaluat on of 50
  long term 50–54
    in angina pectoris 51
    in arteri clerotic heart di ease 51
    bleeding from 39
    in myocardial infarction 51
    previous studies in 50–53
    ri k of hemorrhage v th 53
  oral management of 26
  pre ent status of 40–49
  prophylactic not curative 50
  in recurrent thromboembolism 50
  and se ere hyperten ion 48
  in strokes 49
  in thrombotic d o ders 40
Ant coagulants
  gradual reduction of 39
  natu ally occurring 15–16
  oral 2 -26
    aver ge dosage of 4
    coagulation lefects with 8
    inh bition of prothromb n synthe sis w th 17

specific f c a ulat n facto s 4
vitamin 9
Dental extraction with j rolonge 11 leed ing 39
D agn si l Ifferential in cerebral a cular l sease 46–48
D cumarol 4 9 1 11 1 24 41
and accelerato r glol ulin 5
action of on cl tting factors 2
with vitamin K 18
c agulati n defects caused by 1
contrain l cations to 24
effect of on circulating fibrin gen
on vitamin K in the l ver 2
elimination of 1
l ng term coagulation lefects be cause of 3
recurrent thromboembol m be cause of 3
sens tivity to 1 24
to ic man festations of 24
Dipaxin 17 24 25

Embol sm
arterial 45–48
pulmonary 40–41
anticoagulant therap in 40

Factor dep es on 33
Fibrin
action of fil rinolysis on 90
con er on of fibrin gen to 6 13
electr phoretic patterns of 5
format on 6–
tage three reaction in 6
velocity of 1
reaction of to fib noly in 5
Fibrinogen
action of fil nol in on 90
c culating eff ct of Dicumarol on
effect of heparin on
con er on of to f br n 6 13
defic ency n
determ nation 14
electrophoreti p tterns of 6
level depres io n 8
react n of to fib inol n 5

Fibrin lysin 1 20 8 53– J
action of
on fibrin 90
on f brin gen 20
ctivation of 55
lepressant in fibrin gen 8
letermination 14
electrophoretic patterns of 56
human 20
inactivation of 58
in intra asc lar thrombo s 90
l si of clots with 53– J
reaction
of f brin to 55
of fibrinogen to 55
systemat c reactions to 6 68
toxic reactions of 90
Fibrinolytic agents 19
Fibrinolyt c reactions 19

Hageman factor 4 12 1 33
Hedulin 1 24
depression of prothrombin time with 25
disadvantages of ?
Hemoper card um in myocardial n farction 30
Hemophil a
clotting n 3
thrombopla t n generation in 11
Hemorrhage
cerebral due to ant coagulant ther py 31
incidence of with ant coagulants 90
ri k of 53–54
in antithrombotic therapy 21
with long term ant coagulant ther apy 53
use of protamine sulfate in 28
tolu d ne blue in 28
Hepar n 8 13 16 21 28 41
action of 16
administrat on of 16
contraind cat ons to 22
disad antages of 17
dosage 22
effect of on circulat ng fibrinogen

use of in arterial occlusion 4
Antifibrinolysin 19
Antihemophilic globulin in clotting 3
Antistreptokinase 19
Antithrombin
inactivation of thrombin with 15
synergistic action with heparin 15
Antithrombotic agents clinical use of 21–39
therapeutic 16–20
Antithrombotic reactions 15–20
Antithrombotic therapy aim of 21
Antitrypsin factors in plasma 20
Arterial occlusion 55
Arteriosclerosis coronary and blood coagulation 54
Arteriosclerotic heart disease long term anticoagulant therapy in 51
Aspirin action of with anticoagulants 17

Bleeding due to long term anticoagulants 39
effect of vitamin K on 33
prolonged from *dental extraction 39*
time 9
test for 9
Blood hemophilic clotting of 3
Blood clot definition of 5
effect of trypsin on 59
see also Clot Clotting
Blood coagulation and coronary arteriosclerosis 54
mechanism of 1–
see also Coagulation

Capillary fragility test for 9
Cerebral hemorrhage due to anticoagulant therapy 31
Cerebral vascular diseas 48–49
anticoagulant *therapy in 48*
differential diagnosis in 46 48
Christmas disease 3
Chymotrypsin 8 60–61
activation of 60
contraindications to use of 60
Classification of patients with myocardial infarction 43–44
Clot
lysis of 16
retraction 10
Clotting
abnormal correction of 3
antihemophilic globulin in 3
delayed with prolonged prothrombin time 3?
factors action of Dicumarol on ?
of hemophilic blood 3
intravascular mechanism of 15
tests 8–14
time 10
screening test for 10
Coagulation
and coronary arteriosclerosis 54
defects caused by oral anticoagulants 8
from long term Dicumarol 3
factors congenital deficiency of 3
after hepatic lobectomy 17
plasma defect in 10
reduction of in liver disease 17
specific deficiencies of 4
prophylactic prevention of 16
reactions ?
inhibitors of 15–16
Convertin 4
Coronary arteriosclerosis and blood coagulation 54
Coumadin 17 21
effect of 21
Coumarins 17 [illegible]
Cumopyran 17 21
therapeutic effect of ?5

Dandone 25
Deficiencie
congenital
of coagulation factors [illegible]
[illegible]
fibrinogen [illegible]
[illegible]
platelet 10

Serum
acceleator globulin 4
defects in 11
fibrinolytic activity in 14
normal coagulation factors ad orbed from 10
plasma throml oplastin comp nent in 3
prothrombin conversion accelerator 4 5
determinat on 14
thromboplastic f ctors depressi n of 33
Shock syndrome v ith tryj in injections 20
Sintrom 17 24
SPCA determination 14
Staphylokinase 5,
Steatorrhea 18
Streptokinase 1 19 0 5 59 61–63
activation with 61
clinical use of 62
contraindications to use of 63
Strokes anticoagulant therapy in 49
Stuart factor 4 6 1 17 33

Thrombi intravascular 5
Thrombin
con ersion of prothroml in to 1
definition of 14
formation 5–
stage two eaction in 6 13
gener ition a nount of 14
inactivation with antithromb n 1
Thrombocytopathia 9
Thrombocytopenia 9
Thrombocytosis 9
Tl romboembolism recurrent
ant coagulant therapy in 50
in patient on Dict m rol 3
tendency to ar l 38
Thromboly by v irious enzymes 8– 9
Thrombolytic agents 19
Tl ombolytic therapy 9
Thrombophlebiti 40–41
anticoagulant tl erapy n 40
Thromboplastin
active 6
formation cf
activity loss of 4
formation reagents for 11
generation 2–
in hemophilia 11
test 11
intrinsic 11 1
plasma 11 12
formation of 12
tissue 1 4 11 13
Thrombosis
intrava cular fibrinolysin in 0
venous
Ti ue thromboplastin 1 4 11 13
Toluidine blue in hemorrhage 8
Tourniquet test 9 98
Toxicity
v ith Dicumarol 4
with fibrinolysin 20
Tromexan 17 4
disadvantages of 4
therapeutic effect of 1
Trypsin 1 20 8 9–60
contrain lications to u e of 59
effect of on blo d clotting 59
injections shock syndrome v ith 0

Vas ular disea e cerel ral 48–49
Vitamin
deficiencies 9
K def ciency 18
n liver effect of Dicumarol on 2
in synthes s of prothrombin
use of by liver 17
K action of Dict marol with 18
effect of on bleed ng 33
tablets 21
u se of in hemorrhage 8
Vitamins C and P effect of on bruising 54

Ware and Seegers t o stage prothrombin test 37 112
W rfari sodium 4

oral inactivity of 21
synergistic action of antithrombin with 1ɔ
therapy termination of ‿‿
Hypertension severe and anticoagulant therapy 48

Indandione derivatives 17 24 27
Infarction myocardial acute 41–4ɔ

Liver
disease reduction of coagulation factors in 1⁄
use of vitamin K by 17
Lobectomy hepatic coagulation factors after 17
Lysis of clots 16
with fibrinolysin 63–⁄9

Malnutrition 18
Manchester bedside test for prothrombin 37 10
Marcumar 1⁄ ⁹4
efficacy of 2ɔ
Mephyton use of in hemorrhage 28
Miradon 1⁄ ⁹4 2ɔ
Myocardial infarction
acute 41–4ɔ
classification of patients with 43–44
complications in ⁹9
effect of anticoagulant therapy on 41 43
hemopericardium in 30
long term anticoagulant therapy in ɔ1 ⁹
myocardial rupture in 31

Occlusion arterial 4ɔ–48 5ɔ
use of anticoagulants in 4ɔ
Owren Ware Stragnell one stage prothrombin test 3⁄ 104

Phospholipids 11
effect of on Dicumarol induced purpura 54
substitution of for platelets 2

Plasma
antitrypsin factors in 20
defect of coagulation factor 10
deficient preparation of 10
normal coagulation factors adsorbed from 10
platelet free 1
prothrombin activation of 14
thromboplastin 5 11 1⁹
component deficiency 3
formation of 12
intrinsic 8
Plasminogen 16
Platelets
count 9
deficiency 10
substitution of phospholipid for 2
thrombocytopathic 11
Polysaccharide bacterial pyrogen free 58 61
Pro accelerin 4
Profibrinolysin
activation of 5ɔ ɔ7
conversion of 16
Protamine sulfate in hemorrhage ‿8
Prothrombin
congenital deficiencies in ￼
consumption 12
conversion of to thrombin 1
determination two stage 14
levels during long term anticoagulant therapy 37
synthesis inhibition of 17
with vitamin K ɔ
test of Owren Ware Stragnell 37 104
test of Ware and Seegers 37 112
time 1⁹
depression of with Hedulin ⁹ɔ
normal 13
prolonged 13
and delayed clotting 3
test for 12
Pulmonary embolism 40–41
anticoagulant therapy in 40

Reactions coagulation ⁹
Recalcification ⁹

Serum
accelerato r globul n 4
defects in 11
fibrinolytic activity in 14
normal coagulation factors ad orbed from 10
plasma thrombo[lastin comp neut in 3
prothrombin conversion accelerator 4 5
determination 14
thromboplastic factors depres ion of 33
Shock syndrome with tryp sin injections 20
S ntrom 1 1
SPCA determination 14
Staphylokinase 57
Steatorrhea 18
Streptokinase 15 19 20 5 58 61–63
activation with 61
clin cal use of 62
contrain lications to use of 63
Strokes anticoagulant therapy in 49
Stuart factor 4 6 12 17 33

Thromb intravascular 5
Thrombin
conversion of prothrombin to 1
definition of 14
formation 5 6
stage two reaction in 6 13
generation amount of 14
inactivation with antithromb n 15
Thrombocytopathia 9
Thrombocytopenia 2 9
Thrombocytosis 9
Thromboembol sm recurrent
ant coagulant therapy in 50
in patient on D cumarol 32
tendency toward 38
Thrombolysis by various enzymes 58–59
Thrombolyt c agents 19
Thrombolytic therapy 9
Thrombophlebitis 40–41
anticoagulant therapy in 40
Thromboplastin
active 6
formation of 5
activity loss of 4
formation reagents for 11
generation 2–5
in hemophilia 11
test 11
intrinsic 11 12
plasma 5 11 1
formation of 12
ti sue 1 4 5 11 13
Thrombo is
intravascular fibrinoly in in 0
venous 55
Ti ue thrombo[lastin 1 4 11 13
Tolui line blue in hemorrhage 28
Tourniquet test 9 98
Toxicity
with Dicumarol 1
with fibrinolysin 20
Tromexan 17 24
disadvantages of 21
therapeutic effect of 21
Trypsin 1 0 8 59 60
contraindication to u e of 57
effect of on blood clotting 53
injection shock syndrome with 0

Vascular d ea e cerebral 48–49
Vitamin
deficien ie 9
K defic ency 18
in liver effect of D cumar l on 2
n synthe i of pr thrombin
u e of by liver 1
K action of Dicumarol with 18
effect of on bleeding 33
tablets 21
u e of in hemorrhage 28
Vitamins C and P effect of on bruising 54

Ware and Seegers two stage prothrombin test 37 112
Warfa n sodium 21